PROJECT FINAL REPORT

Grant Agreement number: 310465

Project acronym: MEMBRANENANOPART

Project title: Modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane and function

Funding Scheme:

Period covered: from 1 Jan 2013 to 31 Dec 2015

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MembraneNanoPart

Modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane and function

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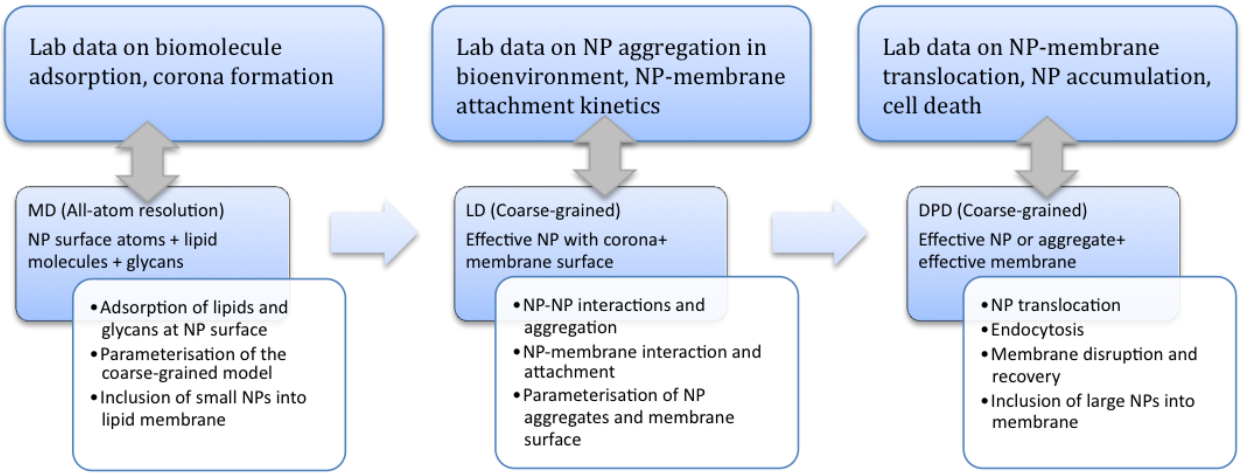
1 Executive Summary

MembraneNanoPart was a three-year EU FP7 project running from 2013 to 2015. It brought together 4 partners from EU countries and Russian Academy of Science. The aim of the project was to develop a methodology of modelling interaction of nanoparticles with biomolecules and cell membranes to enable mechanistic interpretation of nanoparticle toxicity data, provide tools for toxicity assessment, and thus facilitate the production nanomaterials that are safe by design.

MembraneNanoPart programme was motivated by the need in understanding the health risks and mechanisms of appearance of adverse effects associated with the use of engineered nanomaterials. The consortium focussed on the nanoparticle-cell membrane interactions and cytotoxicity effects, while taking into account protein corona of nanoparticles. The principal research tool of the project team was computer simulation of these processes at the molecular level. The consortium followed a mechanistic bottom up modelling approach where the models of biomolecules and cell elements are obtained by systematic coarse-graining of their structure and interactions, and atomistic and molecular level information is preserved in the coarser models so that the material-specific properties can be related to the resulting adverse effects. A chart showing the **MembraneNanoPart** methodology and data flow is shown in the scheme below:



The research performed by **MembraneNanoPart** team identified the challenges in modelling of bionano interface and suggested a number of innovative solutions to address these. The developed models for the first time allowed one to assess the principles of formation of nanoparticle protein corona, rank biomolecules by their binding affinity to nanoparticles, identify the nanoparticle properties responsible for their interaction with plasma proteins and cell membranes, and predict the resulting association and membrane damage. The coarse-grained simulation models were validated against more detailed simulations and experiments. The consortium also developed a scheme for toxicity assessment using diverse biochemical and biomolecular indicators. The project, therefore, prepared a basis for systematic study of the bionano interactions and mechanistic interpretation of nanotoxicological data.

The research and deliverables from the **MembraneNanoPart** project have been disseminated widely both locally, in the countries of respective partners and internationally through journal and conference presentations, Nanosafety Cluster Compendia and meetings. The results were reported to software industry (Dassault Systemes Biovia Ltd.) via the project Advisory Board. Data from the project have been made available through the project website.

2 Summary Description of the Project Context and the Main Objectives

The need

Human health is traditionally associated with research activity in medicine, biology, and biochemical, biophysical and biomedical sciences. However increasingly opportunities for improving health care and protecting citizens from new threats come from new insights in chemistry and physics allied with biomedical sciences. Protecting citizens from health threats is an objective in the EUs health strategy and an opportunity for added value at the European level. Under REACH rules[1], manufacturers, importers and downstream users have to ensure that their nanomaterials do not adversely affect human health or the environment.

Development of nanotechnologies and nanomaterials, on the one hand, provide us with new opportunities for medicine (nanomedicine) in the form of the capacity to diagnose or treat many of the remaining intractable disease classes (viral, genetic, cancer) using the nanoscale agents or tools. On the other hand, it presents a variety of unforeseen risks, as the nanoparticles challenge the immune system of the human body at length scales where it is not well prepared to react. As of now, little is known concerning the health risks of synthetic nanoparticles, however rapid technological progress creating more (and novel) nanomaterials, with not always well understood biological effects, demands urgent action. Even some of the nanomedical systems (nanoscale drug carriers, etc.) themselves have demonstrated unforeseen toxic properties. There are believed to be tens of thousand nanoparticle types under investigation, potentially entering the market, and their intelligent toxicity assessement is a key for protecting the people's heatlh and the enviroment. The understanding of the potential hazards related to nanomaterials will enable manufacturers to quickly screen out particles with physicochemical properties related with a risk, and either develop new particles or re-engineer their products to modify their properties, thereby designing out the risk factors initially, and in the longer term potentially designing the nanoparticles to be safe.

To enable safety by design, a development of mechanistic approach is necessary, where toxicity assessment is based on the understanding of the molecular interactions between nanoscale objects and living systems. Over the last decade, *in vitro* and *in vivo* experiments have produced significant amount of veritable information that can be integrated into theoretical models with the aim of predicting possible health and environmental effects of engineered nanoparticles (NP). However, even the most systematic studies leave the question of precise toxicity mechanisms associated with NPs wide open. An important finding arising from these studies is that the toxic effects can emerge either from membrane damage or from interaction of nanoparticles, once they are inside the cell, with the internal cell machinery. Therefore, an evaluation of possible risks should include an assessment of nanoparticle ability to penetrate, modify, or destroy the cell membrane, or bind and modify key biomolecules. The cell membrane is the junction where foreign objects meet biological tissues, where they challenge the immune system and present a threat to the tissue function. Being selectively permeable, membranes participate in control of the transport of vital substances into and out of cells. Whereas some biomolecules may penetrate or fuse with cell membranes without overt membrane disruption, no *synthetic material* of comparable size has shown this property. Among the factors determining the outcome of NP-membrane interaction the surface properties of nanomaterials play a critical role, which can implicate the membrane glycans or plasma proteins in conditioning NP prior to cell penetration. In addition, the size and shape of the nanoobjects has been found to be important for their fate inside the living organism.

[1] http://ec.europa.eu/enterprise/sectors/chemicals/reach/nanomaterials/index_en.htm

The concept

In this project, we addressed the issues of NP-cell membrane interaction by computer simulations. We proposed to build a predictive approach to the problem of NP toxicity using a quantitatively consistent hierarchy of modelling elements, which connect together the NP-biomolecule interactions, NP-membrane interactions, NP uptake and translocation, going all the way from molecular specificity to the effects on a sub-micron scale. An overview of some critical steps of NP systemic transport, which we covered in our approach, is sketched in Figure 1.

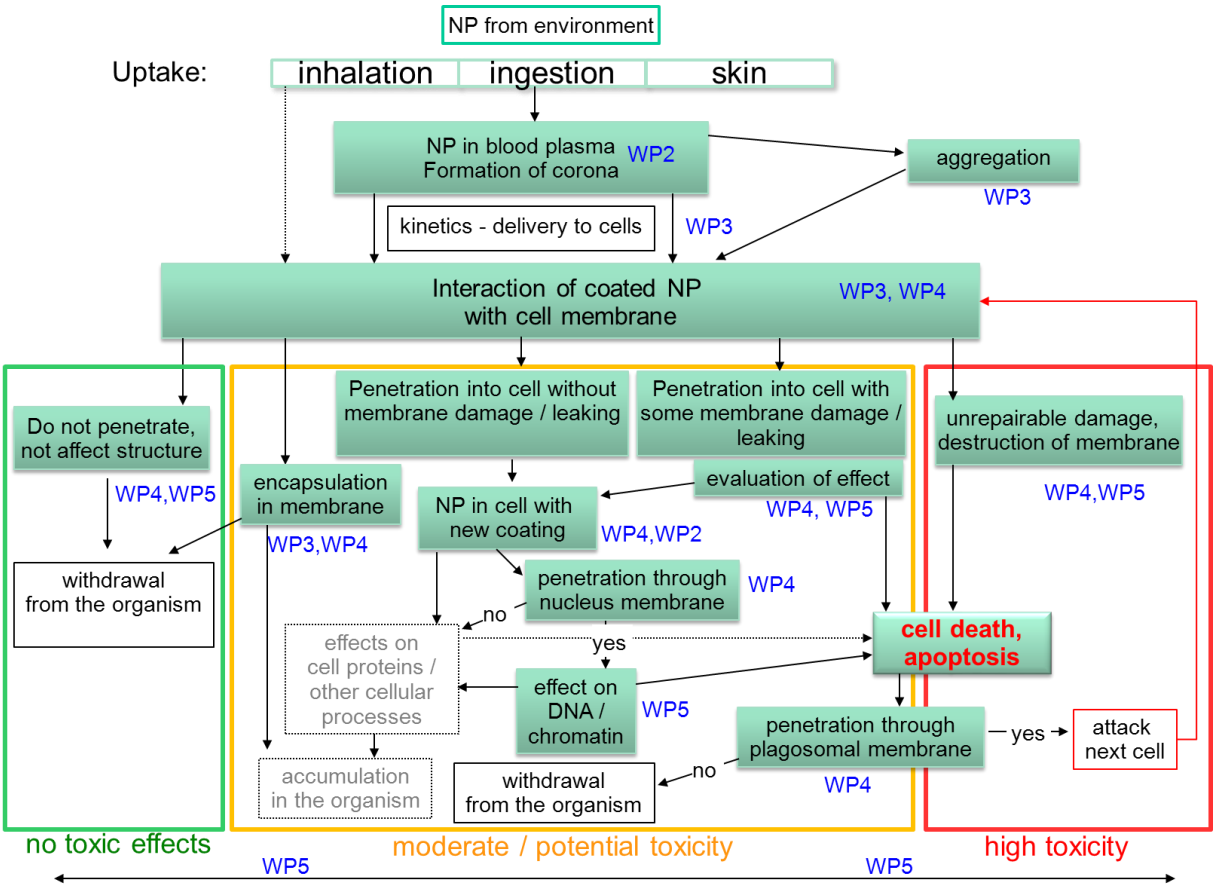


Fig. 1. Overview of the concept: some critical steps of NP systemic transport and Work Packages addressing the modelling of these steps. The questions within the competence of the project are shown by the shaded boxes and labelled by the corresponding work package codes.

Our work packages were built around the need for such elements or modules, and involved:

- modelling the formation of NP corona from blood plasma proteins and lipids
- assessing the role of NP physicochemical properties such as size, shape, hydro-/lipophilicity, surface charge in stability of NP dispersions at physiological conditions, such as blood plasma, and in their systemic transport
- describing complexation of protein-coated or pristine particles with membrane lipids; particle interaction with membrane glycans; the probability of particle inclusion into the membrane
- describing the mechanisms of membrane-bound cytotoxicity: persistent change in the structural, chemical composition and mechanical characteristics of the membrane upon NP inclusion or translocation;

- describing the mechanisms of particle penetration through the membrane via membrane reorganization and disruption, or endocytosis

The objectives

The ultimate **long-term goal** of the topical programme is a development of predictive models for designing and engineering nanomaterials that are safe for humans and environment, so that potential toxic effects could be determined before the material is produced. Development of predictive models is possible only on the basis of **understanding of the underlying molecular mechanisms** of interaction between the nanoparticles and cell constituents. Such understanding needs to be achieved by investigations *in vivo*, *in vitro* and *in silico* by experimentalists and theoreticians working in direct contact with each other.

The main goal of the project was to develop *physically justified models and computational tools to quantitatively describe and understand the molecular mechanisms of nanoparticle-cell membrane interactions*, which we consider to be a crucial point in any predictive model of nanoparticle (NP) toxicity. We attempted to build a self-consistent bottom-up multiscale simulation scheme starting from NP-biomolecule interaction at the atomistic scale using molecular dynamics simulation, and then systematically construct coarse-grained (CG) mesoscale models for simulating the structure and dynamics of the cell membrane perturbed by NPs at the physiologically relevant time and length scales.

In order to achieve the above goal we combined a broad range of molecular simulation methods and algorithms covering multiple time- and length scale relevant for the NP-membrane interactions. We used classical molecular dynamics simulations to study atomistic level of NP-protein and NP-lipid membrane interactions, as well as the effect of NP on the membrane structure. We implemented the idea of the multiscale modelling approach and used the data obtained at the more accurate level of description to systematically construct the CG models describing the same system on larger time and length scales. At the next stage, we used several levels of CG description to describe NPs of different sizes and cell membranes in a consistent manner. We have calculated the energetic map for different NP end points across the membrane down to the cytoplasm, that has led us to prediction of the NP partition, kinetic barriers, translocation rates, and to prediction of associated risks related to the specific toxicity mechanisms. Finally, we have developed a scheme for toxicity assessment using diverse biochemical and biomolecular indicators which enabled a quantitative assessment of the NP integral toxicity from *in-vivo*, *in-vitro* and *in-silico* data. We expect that the results of this research will help us to identify key NP descriptors that determine the outcome of bionano interactions and NP uptake kinetics and thus enable a formulation of QSAR systems based on the mechanisms of action.

3 Main Scientific and Technical Results

Methodological issues were central for this project and we paid special attention to carefully designing the approach. The need for the new methodology was dictated by several issues: (i) relative immaturity of experimental nanotoxicology, which is, despite an enormous number of groups and methods involved, suffers from absence of common toxicity criteria and definitions, suitable nanoparticle descriptors, as well as data validation protocols; (ii) the absence of molecular level understanding of the nanoparticle toxicity mechanisms, which impedes the attempts to relate nanoparticle descriptors to the toxic action; (iii) the need to address realistic, achievable outcomes that can be verified at every point. We attempted to build **a systematic hierarchical approach**, which contain all the intermediate elements needed to bridge between the physicochemical descriptors of nanoparticles and the specific toxic effects. Although we have no possibility to cover all possible situations leading to toxicity, our believe is that the methodology we design and validate will serve as a template for further development of the knowledge base. The developed theoretical schemes, software programs and scripts, as well as data (e.g. bionano interactions potentials and adsorption energies) have been made freely available through the project website.

3.1 Project concept

In this project, we combined various molecular simulation methods and algorithms covering multiple time- and length scale relevant for the NP-membrane interactions. We implemented the idea of the systematic multiscale modelling approach, using the data obtained at the more accurate level of description to systematically construct the CG models describing the same system on larger time and length scales. We used classical molecular dynamics (MD) simulations to study atomistic level of NP-protein and NP-lipid membrane interactions, as well as the effect of NP on the membrane structure. We then employed several levels of CG description and Lattice Boltzmann and Langevin Dynamics simulations reaching to length scales up to hundreds of nanometres in order to describe NPs, NP protein corona, and cell membranes in a consistent manner. Finally, using a calculation of the energy profiles for different NP end points across the membrane we attempted to predict the NP partition, kinetic barriers, translocation rates, and predict the risks related to the specific toxicity mechanisms. In addition to this direct calculations of NP uptake and toxicity, we expect that the results of this research will enable the subsequent formulation of expert QSAR systems based on key nanoparticle descriptors including parameters of bionano interaction. A scheme illustrating the multiscale methodology is shown below.

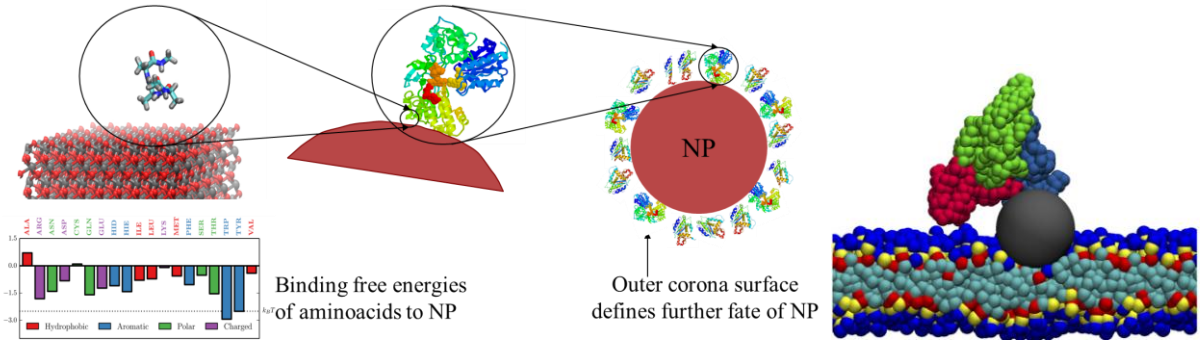


Fig. 2 Left: Scheme of the multiscale simulation technique for measuring the protein adsorption energies and testing the likelihood of key molecular events (NP – nanoparticle). Right: Snapshot from CG simulation of NP-protein complex interacting with lipid bilayer.

List of Work Packages

The project activities have been divided into 7 work packages (WP):

- WP1 dealt with data exchange and acquisition from external sources and provide the material for model parameterisation and validation, such as NP characteristics and cell viability data.
- In WP2, we performed atomistic modelling of the NP surface, blood plasma components, glycans, and lipids. We calculated the NP-protein binding energies, parameterised CG models of the NP-lipid interactions, and CG models for selected components of blood plasma and of the membrane to feed into large scale simulation in WP3-5. We also independently evaluated the NP descriptors to be used in WP5.
- In WP3, we used the CG models of NP, blood plasma, and membrane in CG MD simulations to predict the NP equilibrium state in plasma, and describe the effective nanoparticle, the entity that will be seen by the cell. We also calculated the NP-membrane interaction potentials to be used in WP4-5.
- In WP4, we used atomistic and CG models of NP and membrane to study the possible scenarios of NP-lipid membrane interaction: attachment, translocation, inclusion, envelopment. We calculated the energy barrier profiles for the NP across the membrane and used them for predictive models in WP5.
- In WP5, we studied the aftermath of the NP-membrane interaction and quantified the specific cytotoxicity effects: membrane reorganisation and pore formation. We calculated the NP partition with respect to the membrane and correlated the toxicity with the NP exposure.
- WP6 and WP7 contained dissemination and management tasks.

Below we describe the work packages and the main results achieved within our work.

3.2 WP1: Data acquisition from partner EU projects, quality assurance for data entering the project

WP1 contained three tasks:

- Liaison with ongoing and new EU projects to secure high quality data for use in the atomistic and CG models in WP2-4
- Development of the minimum set of data requirements to support the input data (characterisation data, dispersion and exposure protocols, etc.)
- Liaison with ongoing EU and international databasing projects to ensure that the data is compatible with other databases and search tools

Summary for WP1

We have reviewed existing literature on the toxicity of TiO_2 nanoparticles, where we summarized the main toxicity mechanisms of TiO_2 NP and their relation to the exposure and various dose metrics. The toxicity of TiO_2 has been demonstrated to have inflammogenic, oxidative, and genotoxic consequences. We concluded that mass dose does not well reflect the biologically effective dose for NPs. Other dose metrics, such as surface area combined with surface reactivity or the particles number should be evaluated as superior descriptors of the potential to cause damage. The outcome of biochemical and cytotoxicity studies performed with TiO_2 showed their relation to

physicochemical characteristics of NPs: size and size distribution, surface chemistry (coating), concentration, surface charge, crystalline structure.

On the basis of the literature study we concluded that a major deficiency in studying the mechanisms of nanotoxicity is the characterization of the NP prior to but not after exposure to living cells or animals, so that the actual NP state inside the body is not known. We prepared recommendation to extend NP description in the toxicity studies. We suggested that a proper characterisation should include: size, size distribution, shape, surface area, surface chemistry and properties (surface modification or coating, added functional groups, surface reactivity), crystallinity (crystal type, crystal phase), morphology, solubility, charge, aggregation tendency (particle aggregation/agglomeration), impurities, homogeneity of dispersion. In addition, where possible, the NP descriptors characterising the bionano interactions should be provided: hydration energy, refractive index, ionisation potential, conduction band gap, protein/lipid adsorption energies.

3.3 WP2: Modeling and parameterization of interactions of NP with relevant biomolecules

The research conducted within WP2 was divided into four tasks:

- Establishment of molecular and thermodynamic models for nanoparticle interaction with blood plasma and formation of NP corona
- Establishing of CG models for interaction between NPs and biomolecules
- Validation of the models for corona formation. Direct computation of binding affinities of selected blood plasma proteins to nanoparticles
- Demonstration of the predictive nature of the models: confirmation that the experimental data fits the models for nanoparticle-protein and nanoparticle-protein-membrane

Atomistic models for interactions between NP surfaces and bio- molecules

An accurate force field describing interactions of biomolecules with nanoparticles surface is of crucial importance for reliable prediction of the molecular behaviour using computer simulations. That is why we started with overview and evaluation of the existing potential models for such interactions. As our pilot study we chose TiO_2 since properties of the corresponding nanomaterials are well characterised in literature. A literature survey showed the Matsui-Akaogi (MA) model (Matsui, Akaogi, 1991) to be the most widespread TiO_2 force field based on classical potentials. We carried out simulations of water on TiO_2 surfaces showing that the MA model is strongly hydrophilic and overestimates the TiO_2-water adsorption enthalpy with 75% compared to experimental data. We concluded that a less hydrophilic model was needed to capture TiO_2-water interactions in a more realistic fashion.

For the purpose of parameterization of atomistic model for TiO_2 interactions with water and biomolecules, we have implemented Force Balance Method (Wang et al., 2014), which is a flexible framework for optimization of force field parameters to reference data, which may be both of experimental and theoretical origin. For theoretical reference data we have carried out *ab initio* simulations of a water molecule at (100) and (110) TiO_2 rutile surfaces. As experimental reference data we used crystal structure data as well as adsorption enthalpies of water. In this way we developed two models for TiO_2 material, bonded (with material atoms connected by permanent bonds) and non-bonded, allowing to the structure of material to fit the environment. Both models outperform the MA model for the structure (lattice) and thermodynamic (enthalpy) data. Given the extra parameters in the bonded model it is best in reproducing the target data and can be recommended to use for modeling TiO_2 surfaces with well-defined structure, but the non-bonded model is a simple choice that can be preferred to the MA model for interfacial simulations with

biomolecules and solvent when the structural organization of the surface atom is not well known (e.g., in small NPs).

From the analysis of literature data for modelling SiO_2 surface we came to conclusion that force field described in paper (Fateme et al., 2014) provides a good description of interactions of this surface with water and biomolecules as it is consistent with quantum-chemical calculations and experimental adsorption data for small peptides. Our test simulations confirmed these conclusions, and we used the force field of Fateme et al. in our modelling of SiO_2 nanomaterials. Also for gold and for CdSe semiconductor nanomaterials we used force field parameters found in the literature (Heinz et al., 2008; Rabani, 2002).

The developed force fields and calculated potentials of mean force are available through the project website.

Thermodynamic models for interactions of biomolecules with NP surfaces. Computations of adsorption free energies

Within a simple ideal adsorbed solution theory, competitive binding of proteins to a NM surface can be evaluated from the adsorption free energies computed as a sum of adsorption free energies of aminoacids which are in contact with the surface in each specific mode of binding. We carried out computations of adsorption free energies of all 20 naturally occurring aminoacid side chain analogues (SCA) at 5 different NM surfaces: TiO_2 (rutile), SiO_2 (quartz and amorphous), Au, and CdSe, immersed in aqueous solution. A typical simulation setup for a TiO_2 rutile surface, and computed free energies are shown in Fig. 3.

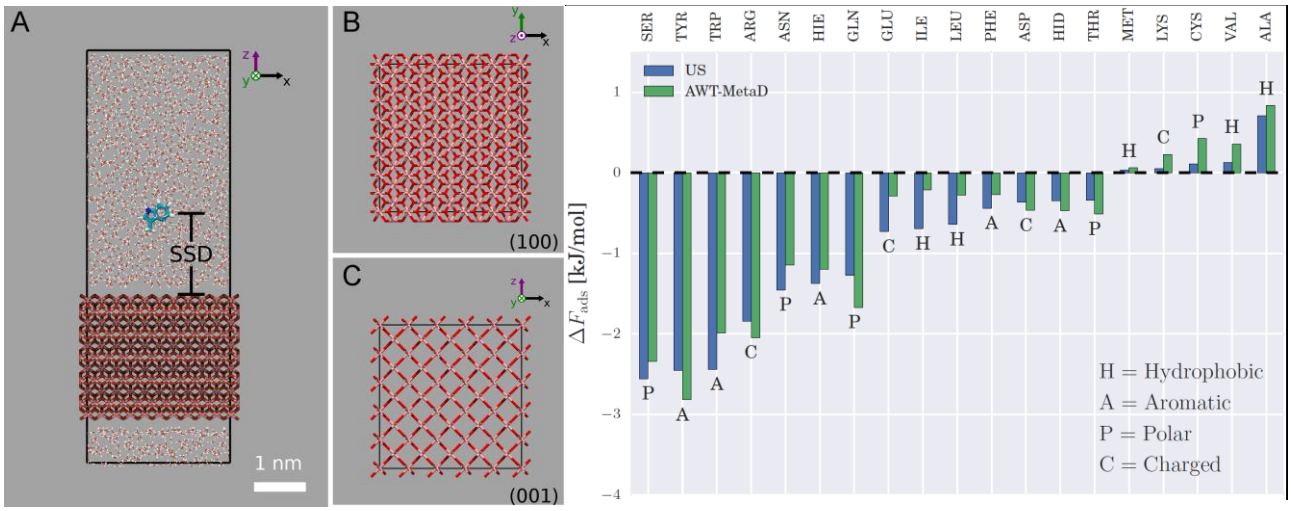


Fig. 3. Left: (A) The simulated system is a TiO_2(100) slab (drawn with sticks), periodic in the (x,y)-directions, and with its normal vector oriented along the z-axis. A side chain analogue (SCA) molecule is placed above the TiO_2 slab, and the surface separation distance (SSD) is defined by the z-component of the distance vector between the centres-of-mass (COMs) of the TiO_2 surface atoms and the SCA. The rest of the simulation box is filled with randomly placed water molecules (drawn with lines). (B) Side-view of TiO_2 along the (100) direction. (C) Side-view of TiO_2 along the (001) direction (lattice planes are denoted by (hkl) Miller indices.)
Right: SCAs adsorption free energies computed using umbrella sampling approach (blue) and metadynamics (green).

From Fig. 3 one can conclude that SER, TYR, TRP and ARG are the strongest binders to rutile TiO_2 surface. One can then expect that proteins would adsorb to this surface by the side which

contains more such SCAs, thus knowledge of the SCA adsorption energies will allows to make qualitative conclusions on composition and structure of the protein corona.

Similar calculations have been carried out for other considered NP surfaces, and for lipid fragments at these surfaces. The computed adsorption free energies determine physical molecular level characteristic of NP-biomolecular interactions, thus they can be used as descriptors in future "molecular mechanisms aware" QSAR schemes aimed at prediction of toxicity of new nanomaterials.

Development of coarse-grained models

Full atomistic modelling of NPs of sizes tens – hundreds nm, together with surrounding aqueous media and biomolecules is not realistic at the current level of computer power, thereafter use of CG models becoming necessary. According to the CG approach, groups of atoms are united in single interaction site (for example one aminoacid of a protein is presented as a single particle), and the solvent modelled implicitly as a continuum media. This reduces greatly the number of degrees of freedom to deal with during the modelling, and allow to simulate large systems on hundreds nm length scale. Significant efforts, however, need to be invested to compute effective CG potentials, which substitute atomistic force field in CG simulations.

In our work, we have used two approaches to build CG potentials. For interactions of proteins and lipids with NP surface we defined CG potentials as potentials of mean force for a separate aminoacid SCA or a lipid fragment near an NP surface. The general simulation setup was the same as for calculation of aminoacid adsorption free energies (Fig. 3), and such potentials were determined for all 20 naturally occurring aminoacids, as well as for phospholipids fragments at 5 considered NP surfaces: TiO_2 (rutile), SiO_2 (quartz and amorphous), Au, and CdSe. An example of SCAs PMF at Au surface is shown in Fig. 4. These CG potentials were used in CG simulations of protein binding to NP surface and corona formation.

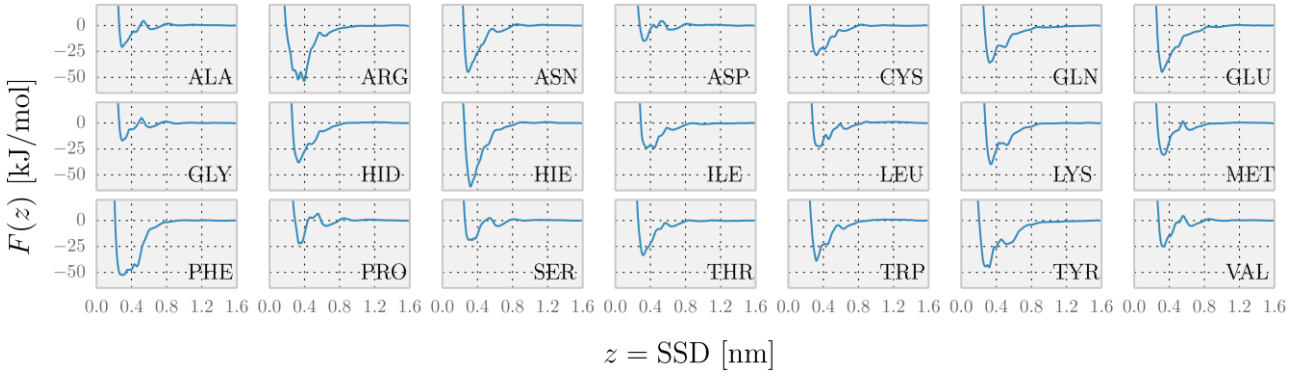


Fig. 4: Potentials of mean force of aminoacid side chain analogues at Au(100) surface.

For description of biomembrane (presented as a lipid bilayer) we have used CG potentials deduced from atomistic simulations of lipids in water using the inverse Monte Carlo (IMC) approach (Lyubartsev et al., 2015). Within the IMC approach, radial distribution functions and other structural properties of the system obtained in atomistic simulations, are used to derive effective CG potential which reproduce exactly the same structural properties in CG representation. We have derived a set of CG potentials for typical components of lipid bilayers including phosphatidylcholine (PC) and phosphatidylserine (PS) lipids with saturated and unsaturated tails and cholesterol (Chol), see Fig. 5 for 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylserine (DOPS) lipids. These components allow to create CG presentations of a large variety of lipid bilayers of different composition.

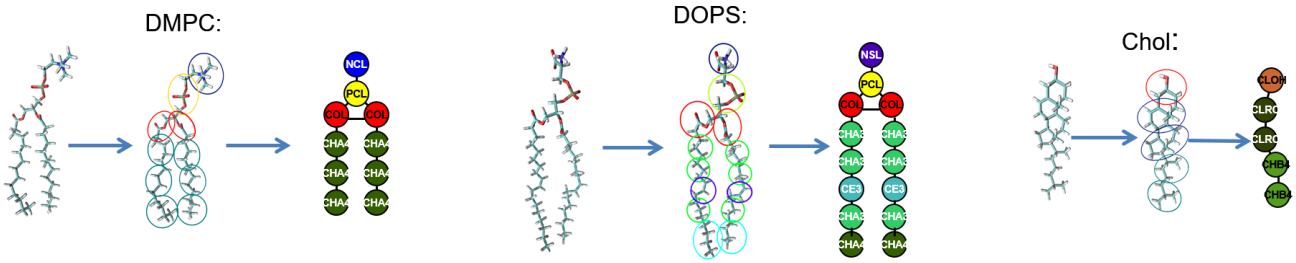


Fig. 5: Coarse-grained representations of typical components of biological membranes: DMPC and DOPS lipids as well as cholesterol.

Validation of atomistic and coarse-grained models and confirmation of their predictive nature

Validation of the developed models followed two main lines: the internal consistency of the obtained data (for example, to ensure that CG simulations produce the same results as atomistic simulations for test systems), and with experimental data in cases when such data are available. Particularly, we have carried out atomistic and CG simulations of ubiquitin protein at TiO_2 and gold surfaces and found that it binds with the similar orientation. We have also carried out CG and atomistic simulations of a number of lipid bilayers with lipids not used for parameterization of the effective potentials and found good agreement.

Validation of the model by experimental data and demonstration of their predictive character was partially hindered by the lack of adsorption data, which are directly comparable with simulations. At the moment of writing this report there are still no experimentally determined adsorption constants (or free energies) available in the literature, or from the other projects, of protein adsorption to inorganic nanoparticles. Arguably, due to the strong adhesion of proteins to solid surfaces, which makes protein binding irreversible on experimental time scales, it is ridden with difficulties to extract adsorption free energies using Langmuir isotherms. We have therefore chosen adsorption data for small peptides and biomolecular fragments available in the literature for comparison to our calculations on TiO_2 and SiO_2. We have compared our atomistic data to available experimental data on fragments and small peptides and have found fair agreement, showing that the models we have employed can predict molecular mechanisms for biomolecular adsorption on inorganic nanoparticles.

Summary for WP2

The main conclusions from the WP2 can be formulated as follows:

- We have developed a protocol and associated software tools for computation of adsorption free energies of aminoacids to NP surfaces. The mechanism to validate and, if necessary to optimize the force fields for such computations is developed and tested for the TiO_2 and SiO_2 surfaces. Production simulations of adsorption free energies have been carried out for 5 NP surfaces. The developed protocol allow for automatized computation of free energies for different NP which can be used as descriptors of NP surfaces to be put in "molecular mechanisms-aware" QSAR schemes. This aim will be pursued in our next Horison2020 project SmartNanoTox.
- We developed a method of systematic construction of CG models that can be used to calculate the adsorption energies for the interactions between proteins or lipids and hydrophobic, hydrophilic or charged NPs.
- The CG model parameterized from atomistic simulation of saturated and unsaturated lipids and cholesterol to simulate multicomponent biomembranes and their interaction with nanoparticles was developed.
- Our results obtained by CG modeling with generic aminoacid-NP potentials showed

furthermore that the charge has a small effect on the adsorption energies in comparison to Van der Waals interactions between the residues and the surface. We also find that the charge of the NP does not influence much the orientation, in which the proteins prefer to adsorb. On the other hand, the size of the NP has a big effect on the adsorption energy maps, as the size of the NP determine the sections of the protein that can interact with the surface.

- In general, bigger proteins adsorb stronger on the inorganic surfaces, even for small NPs. This is a common experimental observation and with our method we were able to qualitatively predict the composition of the NP-protein corona (at least for the long-time dynamics) based on the calculated adsorption energies.

3.4 WP3: Modeling of NP-NP interaction and aggregation, NP interaction with cell membrane, evaluation of kinetic barriers for NP translocation

WP3's work was divided into four tasks:

- Modelling NP-NP interaction in plasma
- Modelling NP aggregation and equilibrium cluster size distribution
- Modelling the NP-membrane interaction
- Demonstration of the predictive nature of the mesoscale model of NP-NP and NP-membrane interaction

Modelling of NP-NP interaction in plasma

We have developed and tested a mesoscale model of nanoparticle-nanoparticle interaction in blood plasma, i.e. in presence of plasma proteins and at physiological conditions. Our systematic coarse-graining approach involved three steps:

- construction of a united-atom model of proteins, based on Protein Databank (PDB) structures
- united-atom simulation of the protein globule – NP interaction and calculation of adsorption energies with full account of the size and shape of both proteins and NPs
- mesoscale modelling on NPs dispersed in blood plasma with adsorption energies and mobilities calculated in the united-atom model

Our model of the direct nanoparticle interaction is based on the standard Derjaguin–Landau-Verwey-Overbeek (DLVO) theory and uses only central forces between NPs. It is well known in colloid science that van der Waals attractions leads to aggregation of the most known colloids in aqueous media, therefore special efforts are made to stabilize the dispersions using chemically bound coatings (citric acid, carboxylic acid are the most common examples). As we were interested in assessing the cytotoxicity of NPs *in vivo*, we limited our calculations to physiological conditions: temperature of 310 K and physiological ionic strength of 100 mmol/L, although we performed also tests of stability of the same materials at low ionic strengths (pure water) where corresponding data were available. We have systematically modelled dispersions of NP from 5 main groups of nanomaterials at physiological conditions. The interactions were evaluated using DLVO potential of the form

$$U_{\mathrm{DLVO}}(r) = U_{\mathrm{HC}}(r) + U_{\mathrm{el}}(r) + U_{\mathrm{vdW}}(r)$$

consisting of excluded volume (HC), electrostatic (el) and van der Waals (vdW) interactions.

For modelling the NP-protein corona formation and NP-NP interactions mediated by the proteins, we use a more detailed presentation of the NPs and proteins surface. In our approach the NP surface and the plasma proteins are represented by nanoscale structures (united-atom beads) that

reflect their size, shape and surface inhomogeneity. The particle is represented by CG surface sites (SB), and inner sites (IB). The beads are either connected to each other by a spring network or a rigid structure is used. This model gives a good compromise between the structure details of the NP and computational efficiency.

To evaluate the blood plasma and protein-NP interaction, we restricted the change of protein conformation and modelled the proteins as rigid structures. Namely, we obtained the native structure of the protein from PDB, which we coarse-grained by replacing each residue by one spherical bead and added bonds to fix the protein in the native structure. Fig. 6 shows the native structure for α_1-antitrypsin protein (A1A) obtained from the PDB ID: 3NE4 and our CG model.

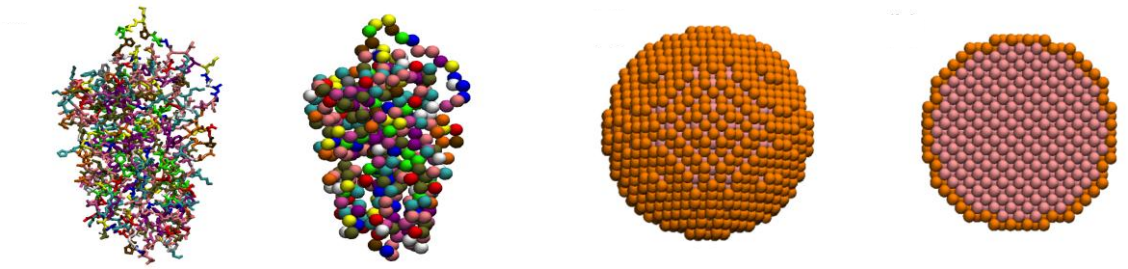


Fig. 6: Structure of the α_1-antitrypsin protein. Left: All-atom structure obtained from the PDB (ID: 3NE4). Middle: United-atom model proposed by MembraneNanoPart project. Right: united atom CG nanoparticle represented with a two-layer model: Surface bead and Inner Beads.

Modelling NP aggregation and equilibrium cluster size distribution

In this task, we attempted to predict the aggregation state of common engineered NPs from the five groups of substances. We found it to be in agreement with literature data for the corresponding NPs and predicted the cluster size for new common engineered nanomaterials.

In case we started from a dispersion of primary NPs, we observed stable or unstable nanoparticle dispersions at physiological conditions, depending on the NP size, surface charge, and core material. We found that surface charge density of 0.05 C/m^2 (or zeta potential of about 50 mV) is sufficient to stabilize NP of any type, while 0.02 C/m^2 (or zeta potential of about 20 mV) can stabilize only dispersions of light materials. This observation is in agreement with previous reports, showing that chemical coatings leading to NP charging up to 30 mV to 50 mV can stabilize the dispersions.

We observed differences in aggregation behavior between metal / metal oxide NPs and carbon-based /polymeric particles on the other side. A higher Hamaker constant (stronger van der Waals attraction) makes the metal containing NPs more prone to aggregation. We observed instability at low surface charge as well as reversible aggregation in the secondary minimum. The semiconductor NPs show intermediate properties. CdSe particles are closer in properties to metal oxides. The maximum NP size, with which the dispersion remains stable at charge density of 0.02 C/m^2 depends on the material: for TiO_2 the aggregation starts at radius R = 2.5 nm, for gold and silver – at 2 nm, for polystyrene – at 25 nm, CdSe – 5 nm, carbon NPs are unstable at all sizes, SiO_2 are stable at least up to 110 nm radius.

Dispersions of very small nanoparticles of sizes below 5 nm can avoid large-scale aggregation due to weak absolute attraction and high entropy even if their surface charge is very high.

Modelling the NP-membrane interactions

We further tried to understand what happens to the NP in blood plasma and how it reaches the cell membrane. Any model that seeks to accurately predict the uptake of NPs from the blood stream must consider that before reaching the cell membrane these materials must overcome an

endothelial protective layer, which is known as the endothelial glycocalyx layer (EGL). This layer is composed of glycocalyx brush, which not only regulates the blood flow but also the material transport along the blood vessels and through the membrane. In this task, we assessed the EGL permeability function, which has the crucial importance for nanotoxicology as the EGL acts as a filter, which selects the NPs that finally will reach the cell membrane and translocate into the cell. The EGL layer makes an impenetrable barrier for NPs with size exceeding 10 nm. Large NPs have to be translocated via active processes such as endocytosis or phagocytosis, while smaller ones can reach the lipid membrane by diffusion.

We then studied the interaction of NPs with the lipid membrane of the cell for the particle sizes that are more likely to overcome the protective barrier of the glycocalyx. We found that a model, where the NP-aminoacid interaction is parameterized in MD simulations with a short cut-off fails to predict correctly the interaction of larger NPs with the lipids. To amend the problem, a two-layer CG model of interaction of NP with lipid bilayer has been developed. The model treats differently surface and bulk atoms of the nanomaterial. The surface layer takes into account hydration of the NPs and the lipids, while the bulk beads include only dispersion (van der Waals) attraction. The two-layer model reproduces the results of atomistic simulations for adsorption of small NPs on the bilayers and predicts that gold NPs get wrapped by lipids already at NP sized of a few nm, small TiO_2 NPs get weakly adsorbed, while silica NPs of 1.5 nm size are repelled from the lipid headgroups but can penetrate the bilayer and stay inside it.

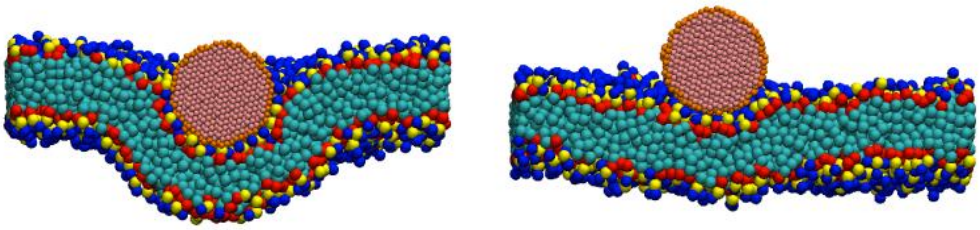


Fig. 7: Attachment of Gold (left) and TiO_2 (right) NPs on a DMPC lipid bilayer. The particle radii are R = 2.5 nm. The surface and inner beads of the NP are marked by different colours.

The main limitation of the common software packages such as GROMACS for this research is the inability to efficiently handle long-range interactions in CG models, which are necessary to model larger NPs in solution or at membranes. We concluded that further work is needed to improve the methodology.

Demonstration of the predictive nature of the mesoscale model of NP-NP and NP-membrane interaction

We used all-atom simulations and experimental data to validate the CG model for calculation the adsorption energies for the interactions between proteins and surfaces and predict the formation of a NP protein corona. As a test case, we studied adsorption of Ubiquitin (Ubi) on a flat TiO_2 surface. We used for the structure of the Ubi the PDB ID file 1Ubi and assumed that the protein is rigid and assigned one bead per residue. The interaction potentials of mean force between the 20 different residues and the surface were obtained by full atomistic simulations of the adsorption of each of the 20 amino acids and then performing metadynamics simulations in WP2. The all-atom MD simulations have confirmed that (i) the protein does not change conformation in contact with the NP and (ii) most of the preferred orientations predicted in the CG model agree with the all-atom MD results, where MD finds stable orientations as well.

We also proposed a second level of coarse-graining of the protein solution to study the formation of the NP-protein corona (Fig. 8). We used the method to study the competitive adsorption of HSA and Fib onto Silica NPs. Our methodology included hydrodynamic interactions

(HI), and we found that their effect on the adsorption kinetics does not change the qualitative picture at low concentrations. The quantitative effect of HI is reflected in the smaller maximum number of adsorbed HSA molecules on the nanoparticles even in the dilute regime. We envision that our methodology can give valuable information on the importance of cooperative effects and conformational changes on the dynamic of the adsorption.

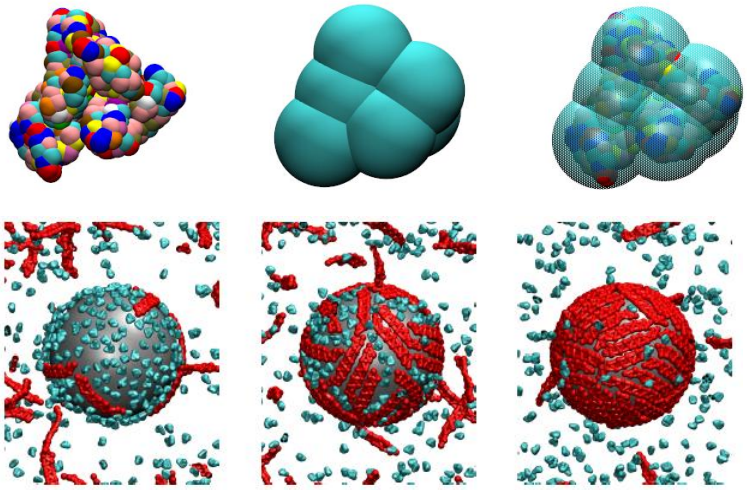


Fig. 8: Top: An example of second coarse-graining of an HSA protein: united-atom to 6 bead united-aminoacid presentation. Bottom: Snapshots of the competitive adsorption of HSA (shown in cyan colour) and Fib (shown in red colour). (left) A snapshot taken when HSA shows its maximum adsorption (middle) Snapshot taken when HSA and Fib have around half of their maximum adsorption numbers. (right) Snapshot taken when Fib replaces HSA.

The rigid-body model of a protein that we suggested can be justified at least for small proteins as no unfolding happens upon adsorption. In general, the CG model we proposed has predictive power in what regards the adsorption energies (to large extent) and preferred orientations (to a lesser extent) as its results are consistent with those of full atomistic simulations. The CG model gives a correct prediction of the relative affinity of common plasma proteins to the NP surface as verified by comparison to experimental data from literature. We proposed that a more accurate CG model can be directly obtained from our methodology by modifying the calculation of the adsorption energies. Another improvement can be achieved by adding internal degrees of freedom (hinges and soft springs) to non-compact proteins like Fibrinogen.

We calculated binding energies for six common plasma proteins: human serum albumin (HSA), Human Serum Albumin (HAS), α_1-antitrypsin (A1A), α_2-macroglobulin (A2M), Fibrinogen (Fib), Transferrin (Tra), Immunoglobulin G (IgG), which represent the groups making over 90% of plasma protein. Ranking of the proteins by adsorption energy on negatively charged hydrophobic particles of various sizes is shown in Table 1. The CG models and the adsorption energies are available fro download from the project website.

Radius [nm]	Ranking					
5	A2M	IgG	Fib	Tra	A1A	HSA
10	A2M	Fib	IgG	Tra	HSA	A1A
15	A2M	Fib	IgG	Tra	A1A	HSA
20	A2M	Fib	IgG	Tra	A1A	HSA
30	Fib	A2M	IgG	Tra	A1A	HSA
40	Fib	A2M	IgG	Tra	A1A	HSA
50	Fib	A2M	IgG	Tra	A1A	HSA
60	Fib	IgG	A2M	Tra	A1A	HSA
80	Fib	igG	A2M	Tra	A1A	HSA
100	Fib	IgG	A2M	Tra	A1A	HSA
200	Fib	IgG	A2M	Tra	A1A	HSA
300	Fib	IgG	A2M	Tra	A1A	HSA
500	Fib	IgG	A2M	Tra	A1A	HSA

Table 1. Ranking of the adsorption energies of plasma proteins on a negatively charged hydrophobic NP. For each NP radius, the proteins are sorted from left (stronger adsorption) to right (weaker adsorption) by their value of adsorption energy.

Summary for WP3

We have developed and validated CG models of proteins and NPs, suitable for modelling of formation of NP protein corona and of interaction of NPs with each other and with lipid membranes. We predicted protein binding affinities, particle-membrane interactions, and NP aggregation behaviour, which is consistent with relevant experiments and more detailed simulations. In particular, we have shown that gold and TiO_2 NPs interact with each other and with biomolecules much stronger that silica, carbon, and other light materials because of stronger dispersion attractions. Moreover, we estimated the protein adsorption energies on NPs and found that they are not affected much by NP charge but rather by NP and protein size and shape. Using our CG model, we ranked proteins by their binding affinity and predicted the adsorption kinetics. The developed simulation method provides much advantage in the computational speed, giving an at least 100-fold acceleration over atomistic MD, while giving a higher resolution as compared to mean-field or more coarse-grained models.

Although we were able to test elements of the model separately, a full integration of the methods was not possible for large NPs with or without protein corona and in contact with the bilayer membranes. This was caused by the inability of common simulation packages to handle all elements of the simulation (lipids, NP, protein) and satisfy all the requirements (presence of charges, large interaction cut-off, constant pressure ensemble) at the same time. One will be in a position to perform simulations of the full NP-protein-lipid systems for NP of over 10 nm size once these problems are resolved.

3.5 WP4: Modelling NP-membrane translocation kinetics, assessment of NP effect on the membrane, connection to cytotoxicity

WP4's work was divided into four tasks:

- Preparation and testing of a model of a cell membrane and cell nucleus membrane
- Penetration of small NPs with size under 10 nm through the cell membrane
- Interaction and penetration of large NPs through the cell membrane
- Construction of a predictive model for the NP penetration

The research undertaken in WP4 as described below has provided new insight into the nature of the NP wrapping/endocytosis problem as well as highlighted the limitations of existing atomistic and CG approaches in particular for situations where the model membrane is penetrated by NPs. We developed CG models of lipid membrane constituents and the membrane and studied interactions of NPs with lipid monolayers and bilayers. Existing CG procedures were found to give incorrect bending and surface tension energies with respect to the atomistic simulations. The CG force fields must be improved to be able to handle NP wrapping bi lipids. We suggested a methodology for solving in further work in successor projects. Until then the most promising procedure to determine NP fates on contact with cell membranes is the mean field approach of (Deserno, 2004), as implemented in (Dasgupta et al., 2013). The outputs from WP4 are the CG force fields and models, NP-membrane interaction outcomes for small NPs, a model for evaluation of energy barriers for NP crossing, and prediction whether a NP is wrapped, partial wrapped or not wrapped by lipids at a cell wall. The data and software (MS Excel spreadsheet) can be downloaded from the project website.

In what follows we discuss the possible scenarios for NP penetration (translocation) of a human plasma membrane (the 'barrier') based on our simulation work and theoretical developments of WP4.

Modes of nanoparticle translocation

As a first step we distinguished between passive and activated transport. That is, NPs may cross the barrier by some mechanism requiring the cell to do work (expending energy, endocytosis) or passively (the cell does no work). Our project was focused on passive transport.

Secondly, for passive transport we distinguished between the scenarios where the NP enters the cell by destroying the barrier (cell wall) and where it translocates the barrier leaving it intact. *In vitro* studies often show extensive cell damage where bare NP surfaces strongly attract lipids removing a portion of the cell wall with obvious toxicological effect (Lesniak et al., 2012). We focus here on barrier translocation.

Finally, we considered the effect of the NP surface on passive translocation. There is an obvious distinction to be made between hydrophilic and hydrophobic NPs. Our simulations have shown the major importance of the strength of the lipid-NP interaction relative to the lipid-water interaction for hydrophilic NPs: if the water is more strongly absorbed than the polar lipid head group, then there are very significant barriers to crossing the membrane (of the order of 100-200 kJ/mol for a 1 nm-diameter TiO_2 NP through a pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) or DPPC/Cholesterol membrane, Fig. 9), increasing linearly with the NP diameter.

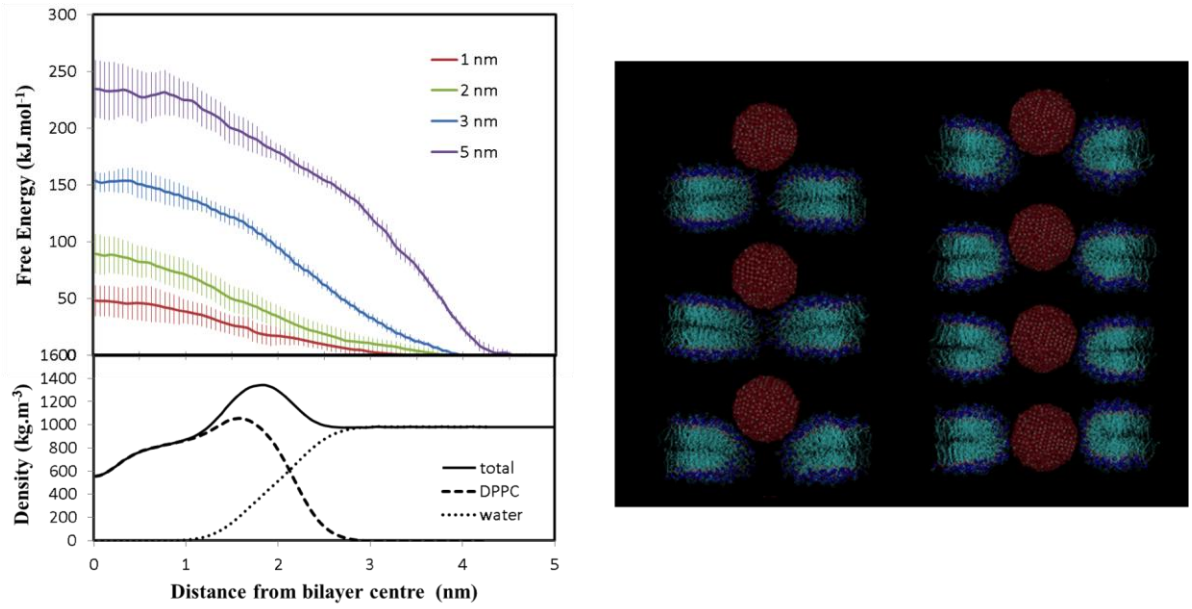


Fig. 9: Free energy profiles for hydrophilic NPs penetrating a DPPC bilayer at room temperature

As one would expect, the hydrophilic particles does not want to penetrate the hydrophobic core of the lipid bilayer forming the membrane and will not attach to the membrane preferring the water phase. If a (residual) pore forms spontaneously in the membrane then the barrier to translocation is smaller.

On the other hand if the lipid head group is more strongly attracted to the NP than water (or there are receptors on the NPs that bond to the head groups) then the strong bonding of the lipid membrane head groups to the NP can result in binding to the membrane (Fig. 10).

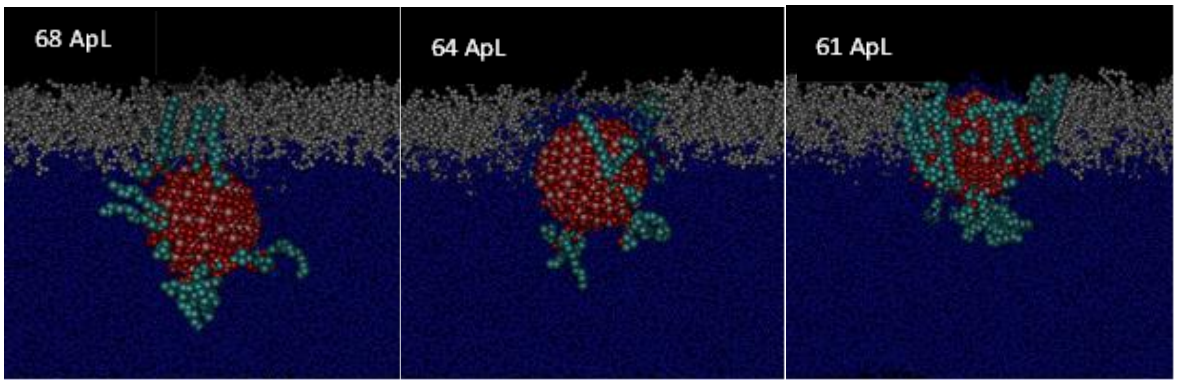


Fig. 10: In atomistic simulations where the NP/lipid interaction is stronger than the NP/water interaction the lipid head groups bind the NP to the headgroup layer. Illustrated here for a water-supported monolayer DPPC layer at three different physiological areas per lipid (ApL), [Å^2].

There are other considerations to do with shape and the presence of edges on the NP surface, which we do not discuss further as we assume that our NPs are spherical.

The free energy of translocation may also depend on the presence of large membrane potentials, in-plane mechanical stress, cell cycle, or cell penetrating peptides and proteins as each of these has the ability to create pores in the cell membrane thereby reducing the free energy required to open a hole big enough to allow translocation. These effects were not within the compass of the present project but should be explored in future.

Experiment shows that many NPs are covered in a protein corona, which determines the biological identity of the NP. The discussion above therefore can be recast as a discussion of the hydrophilic/hydrophobic/specific interactions of the corona.

Identifying which scenario applies to the representatives of the five classes depends then on knowledge of the relative strength of the lipid-NP and water NP interactions in each case. This in turn will depend on the nature of the NP surface, whether water or protein covered, and for the latter the details of the protein (chemistry and conformation). The key conclusion of the MembraneNanoPart project is that the primary identity of the small NPs (1 nm – 3 nm) is less important to the classification of scenarios than the nature of the NP surface and the consequent relative strength of water/lipid/NP surface interactions. On the contrary, for the particles of size greater than 5 nm, the primary identity is decisive. The exact borderline between the surface-dominated scenarios and interior-dominated ones is different for different materials. We found that the transition to the interior-dominated regime is happening at different sizes for different NP materials, where the van der Waals interactions play the most important role. The vdW interactions strength varies as Gold > TiO_2 > SiO_2, CdSe > Carbon, so we expect the transition happening earliest for Gold. Overall, we expect the scenarios of NP binding/wrapping for small Gold, TiO_2, SiO_2, CdSe (for Gold at smallest sizes, TiO_2 larger, etc.), inclusion in the membrane for carbon, polymer, small SiO_2, and non-binding behaviour for intermediate silica.

From our current state of knowledge (based on the work done in MembraneNanoPart) we can predict the potential energy for adsorption of a carbon NP by a lipid membrane (always more favourable for the NP in the membrane than in the water) and the barrier height for hydrophilic particles. For the latter we can estimate the fluxes of such particles across a lipid membrane as below (Fig. 11).

Calculation of free energy barriers to penetration of a lipid bilayer

We have formulated a new toroidal pore free energy model within the opposing forces model (OFM) which can predict the formation free energy for a pore of radius R in a given membrane type given the membrane edge free energy Λ_∞.

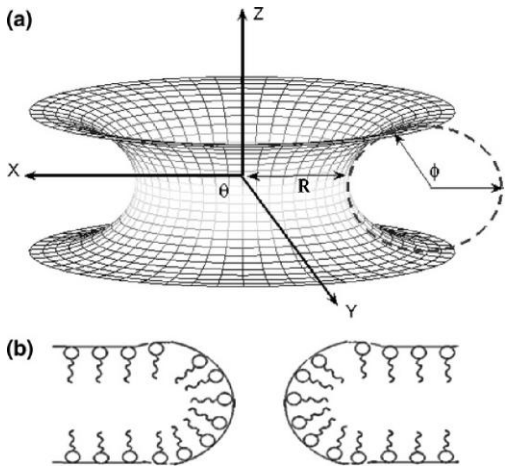


Fig. 11: Geometry of our toroidal pore model for a lipid bilayer (image from Joshi and Hu, 2010)

We have measured this edge free energy using the ribbon method for DPPC membranes and found, $\Lambda_\infty = 26pN$ and used this value to predict free energies required to open a pore to accommodate a NP of radius *R*

$$\Delta G = \frac{2\Lambda_\infty}{9}\left(\frac{4b^2}{\pi R - 2b} + 9\pi R - 30b\right)$$

and compared it to direct free energy simulation finding good agreement as below (Fig. 12)

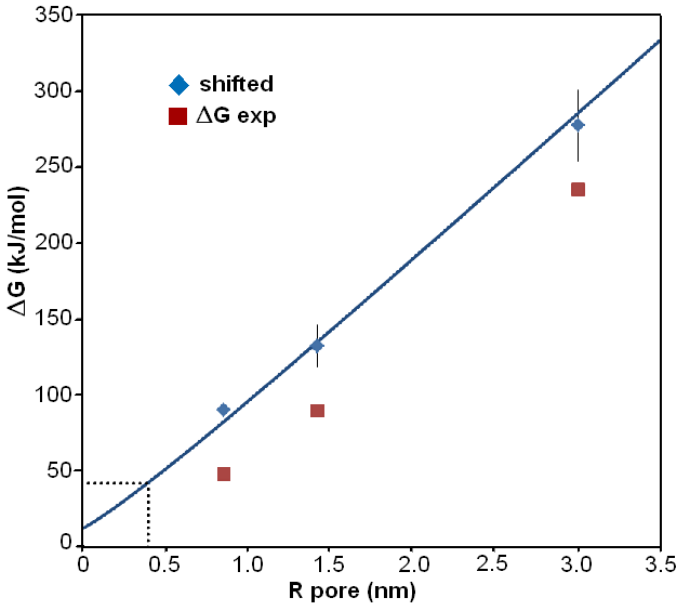


Fig. 12: Free energy required for a hydrophilic NP to penetrate a membrane estimated from molecular dynamics simulations. The free energy of a residual pore of radius R_0 in DPPC is 40 kJ/mol. The blue line is a fit to the free energy required to open a pore of radius R in a DPPC membrane using the equation above. The squares represent the free energy given that a residual pore has formed spontaneously in the membrane prior to NP insertion.

Translocation rate through pores – hydrophilic nanoparticles

We have derived expressions for the rate of translocation of hydrophilic particles into vesicles through pre-existing membrane pores. The translocation is modelled as an activated rate process with the rate, *k*, governed by the Arrhenius equation

$$k = A_c e^{\frac{E_a}{k_B T}}$$

Here E_a represents the activation energy for the translocation process and A_c represents the frequency of collisions between the NP and the pore. Key variables are the vesicle diameter, d_v, the line tension of a straight bilayer edge, Λ_∞, the particle diameter, *d*, and the particle concentration, *N*.

The collision frequency is determined by the diffusion of the particles in solution

$$A_c = 4\pi(d + d_v)DN$$

where *D* is the diffusion coefficient for the particles, given by

$$D = \frac{k_B T}{\mu d}$$

The free energy of formation of a pore in a tension free membrane is derived from the OFM model

$$\Delta G(R) = \frac{2\Lambda_\infty}{9}\left(\frac{4{b_0}^2}{\pi R - 2b_0} + 9\pi R - 30b_0\right)$$

Here b_0 is the bilayer leaflet thickness. R is the outer radius of the pore which is related to the inner radius by $R = r + b_0$. The activation energy is the energy required to expand the pore from the equilibrium size, R_0, to a size sufficient to accommodate the particle, R_p

$$E_a = \Delta G(R_p) - \Delta G(R_0)$$

From our simulations we have determined that typically the expanded pore size is related to the particle size by

$$r_p = \frac{d+1}{2},$$

where the units are in nm. These equations are implemented in an excel workbook containing translocation sheet 2 (http://www.membranenanopart.eu/data/D4.4/barriers.xlsx). Translocation rates are shown in Fig. 13.

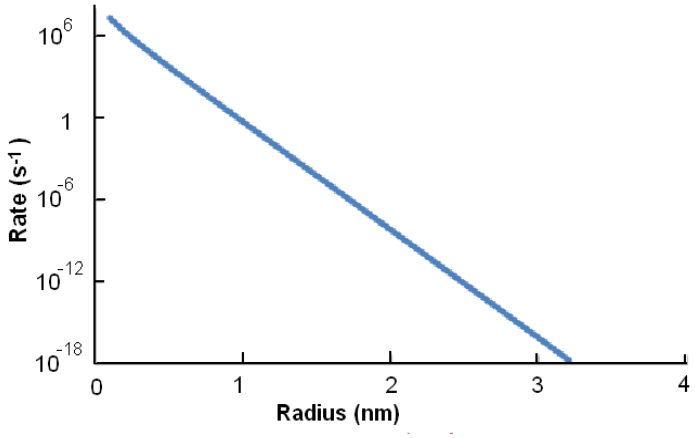


Fig. 13: Translocation rates for hydrophilic NPs calculated in the spreadsheet.

Pathway to a more complete predictive method

The estimates above include surface and edge tension effects but the bending energy component of the non-planar membrane is not treated explicitly. The next stage is to place the simulations in the context of a theoretical description that also includes membrane bending energies and then use the simulations to determine the free parameters in the model and test the assumptions. We start with the continuum model of (Deserno, 2004), which describes the wrapping energy of a NP at a membrane interface in terms of three reduced variables; the reduced energy of the wrapped membrane, the reduced adhesion energy and the reduced bending energy as below

$$\tilde{E} = \frac{E}{\pi\kappa}, \qquad \tilde{\omega} = \frac{2\omega a^2}{\kappa}, \qquad \tilde{\sigma} = \frac{\sigma a^2}{\kappa}$$

where the total energy E comprises an adhesion energy $E_{ad} = 2\pi a^2 z\omega$, with ω the adhesion energy per unit area of NP, a bending energy $E_b = 4\pi z\kappa$, with κ the bending constant and a tension energy $E_T = \pi a^2 z^2 \sigma$, with σ the surface tension and the energy of the free membrane. Minimising this energy leads to the phase diagram below (Fig. 14):

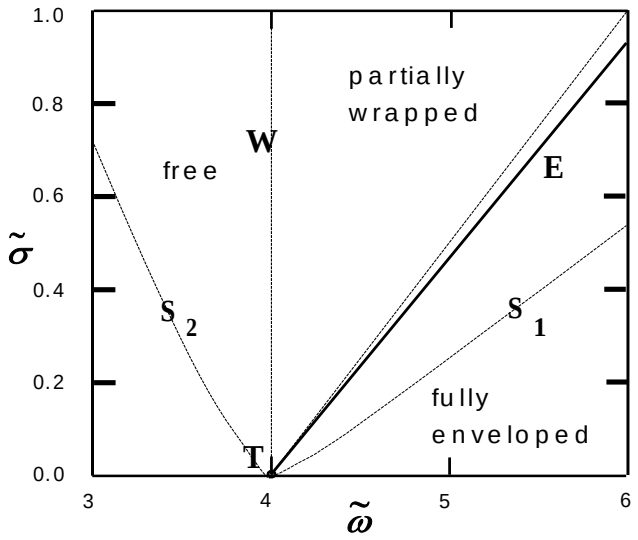


Fig. 14: Wrapping/unwrapping phase diagram (adapted from Bischofberger et al., 2012)

Given the surface tension and the bending tension of the membrane, the membrane bending theory can predict both the end state of the NP in the membrane and any barrier to reaching that state (allowing an estimate of fluxes) as a function of NP size and form the basis for classifying the fate of NPs and NP fluxes, given ω, κ and σ. Its range of applicability can be tested by comparing its predictions with those of atomistic simulations of systems where we know ω, κ and σ.

We have implemented this approach using data from (Dasgupta et al., 2013) to create a spreadsheet that given ω, κ and σ can identify the NP end state (unwrapped, partial wrap and fully wrapped) and the energy barrier if the state is between the line W and the spinodal S_1 (in Fig. 14). This spreadsheet can be downloaded from the project website.

We have tested this model by performing atomistic simulations of gold/lipid bilayer simulations a snapshot of the gold 10 nm NP partially wrapped by a DPPC membrane is shown in Fig. 15.

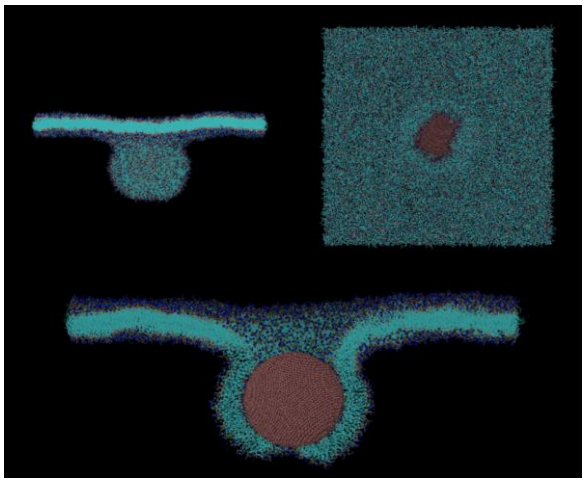


Fig. 15: Gold NP at a DPPC membrane (10 nm Au particle 9216 DMPC Slipids bilayer, fully atomistic, 200 ns, 5,000,000 atoms 580,000 core hours, 66 core years).

These simulations are very challenging, containing 5 million atoms and requiring national (UK) supercomputing facilities. We have an estimate of the bending modulus from an analysis of

the membrane fluctuations as 1.8×10^{-19} J and since our simulations are for a tensionless membrane $\sigma = 0$ (note in the simulations the membrane is only tensionless away from the NP). The Deserno's theory analysis predicts complete wrapping at these conditions. However if the *average* surface tension is non-zero then partial wrapping is expected, which is what we see.

In this project, *for the first time,* we have matched the variation of the surface tension. The difference between the CG and atomistic simulations for the system of Fig. 15 is shown in Fig. 16.

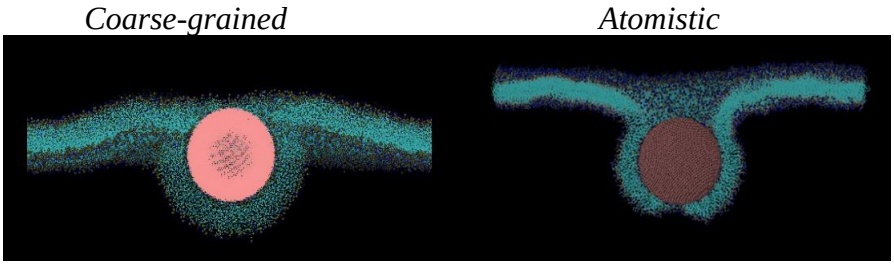


Fig. 16: CG simulation of the system in figure 15 (10 nm Au particle) without matching the bending modulus. The join at the NP is not the same in the two simulations.

From these results we discover that matching the surface tension between atomistic and CG simulations is not enough and that we require a new methodology, which also matches the bending energy. This is a very significant result, which points the way to a new coarse-graining methodology.

Summary for WP4

The research undertaken in WP4 has provided new insight into the nature of the NP wrapping/endocytosis problem as well as the limitations of existing atomistic and CG approaches (system size dependence of most of the key literature results, breakdown of coarse-graining methodologies). We developed schemes for evaluation of the barriers for NP translocation through a lipid bilayer membrane and studied different scenarios of NP interaction with model membranes. The classification system is based on the hydrophilicity of the NP relative to the strength of the NP–lipid interaction. The behaviour of hydrophobic particles, which spontaneously partition in the lipid tail region of the bilayer, was elucidated by simulating spherical fullerene carbon molecules. We showed that free energy change driving the membrane penetration can be readily determined by the difference between the LJ interaction energy of the particle in the water phase and bilayer tail region. The behaviour of hydrophilic particles depends on the relative strength of the NP-lipid interaction. When the water interaction is strong, a water layer is retained around the particle preventing interaction with the lipid bilayer. The energetic barrier to penetration is therefore determined by the pore formation energy.

When the NP-lipid interaction is strong relative to the water interaction the particles adsorb onto the bilayer. The amount of deformation of the bilayer is dependent on the NP-lipid interaction. The small hydrophilic particles (e.g. TiO_2) adsorb with relatively little deformation of the bilayer and the free energy change is due mainly to the enthalpy of adsorption. The gold particles, with a greater lipid interaction energy, adsorb with more bilayer deformation and the free energy change has a larger entropic contribution due to compression of the bilayer at the particle surface.

Building on this work for the future, an approach based on an overarching continuum theory combined with simulation, is recommended. The simulations will provide the input parameters and allow the unambiguous testing of the continuum theories for the fate of the NPs in biological systems including both lung and call membrane lipid interfaces.

Once confirmed, the theory or a more accurate successor can be used with experimental results for membrane tension and bending modulus and NP adhesion energy to rapidly predict the fate and the fluxes associated with different classes of NPs. Where no experimental data exist, simulation has a key role in providing data.

3.6 WP5: Assessment of the toxic effects, development of test suits and prediction tools

WP5 was divided into four tasks:

- Evaluation of the membrane damage following the NP-membrane interaction
- Evaluation of the cell damage following the NP translocation
- Evaluation of genotoxicity from the NP-Chromatin interaction
- Construction of a unified criterion for the risks associated with NP. Validation of the predictive power of the scheme

Review of literature data on nanomaterials toxic effects

We created a database/review on toxicity of TiO_2 NPs (occur in nature mostly as well-known minerals rutile, anatase and brookite), covering the material properties and toxicity mechanisms. It was mentioned that a comprehensive evaluation of the current knowledge regarding the toxic effects induced by TiO_2 and other NPs on organic systems requires considering data for different systems such as respiratory, nervous, dermal and mucosal, cardiovascular, hematopoietic, immunological, renal, musculoskeletal, reproductive systems.

Most studies showed that TiO_2 NPs are toxic to various experimental models, especially when escalating doses are used. However, results obtained from research in cells and animal models cannot be assumed to apply directly to human beings. Such experiments provide the basis to obtain more detail data regarding the hazard of these NPs and to extrapolate evaluations for a correct human risk assessment.

For historical reasons, a focus on the size (and surface area) dependence of TiO_2 NP toxicity has been repeatedly investigated, and confirmation that particle toxicity increases as particle size decreases has been consistent within wide ranging investigations. However, it has become evident that other physicochemical factors are able to contribute to NP toxicity. One major limitation to assess toxicity is the characterization of the NP prior to and after exposure to living cells or animals.

The most relevant physicochemical characteristics of NPs are: size, size distribution, shape (spherical, cubic, triangular, tubular, hyper-branched, needle-like, rod-like, etc.), surface area, surface chemistry and properties (surface modification or coating, added functional groups, surface reactivity), crystallinity (crystal type, crystal phase), morphology, solubility, charge, aggregation tendency (particle aggregation/agglomeration), impurities, homogeneity of dispersions.

All of these properties need to be assessed in order to determine their contribution to toxicity. Generally primary size and often crystal phase is given in literature, whereas other characterization is more random. Therefore it is recommended to do a proper characterization not only based on the manufacturer's specifications, but also of the nanomaterials as applied in the test systems. Due to the lack of appropriate methods to determine the physicochemical nature of NPs in biological systems, the exact nature of NP toxicity is not fully described or understood at this time.

The exposure method, dose administered, species used, cell type under investigation and light conditions also have the potential to impact on the toxicity of TiO_2 and other NPs, indicating that the experimental set up is also very influential to the toxicological observations.

The toxicity of TiO_2 has been demonstrated to have inflammogenic, oxidative, and genotoxic consequences, with these endpoints considered to be inherently linked. Cytotoxicity is also a common end point that is evaluated within studies. The ability of NPs to exert toxicity at a variety of target sites is reliant on their transfer into blood. Accordingly, investigations into the toxicity of TiO_2 via specific routes of delivery, or at particular cell and organ targets, are often insufficient in number to make definite conclusions about NP behaviour.

A quantitative criterion for irreparable membrane damage caused by NP-membrane interactions and NP translocation

We modelled C_{60} NPs in contact with a DMPC lipid membrane and predicted NP uptake and change of membrane properties upon increase of concentration of inserted NPs. The toxicity indicators were evaluated. The indicators show an increase of the membrane damage as a function of NP concentration. Fig. 17 shows snapshots for a side view for 2 NP concentrations: 16 C_{60} and 64 C_{60} molecules. In our simulations, the NPs aggregated in solution and then penetrated the bilayer in a few nanoseconds.

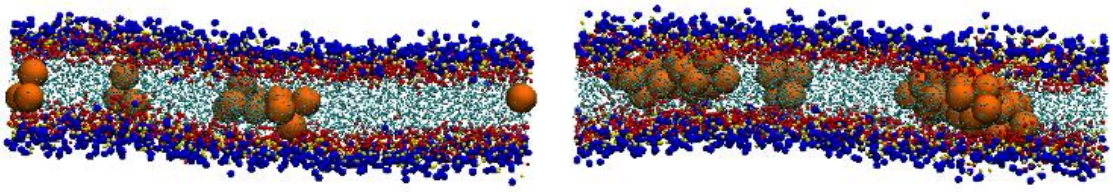


Fig. 17: Simulation snapshots (side views) for two concentrations of NPs: 16 C_{60} (left) and 64 C_{60} (right) molecules inside DMPC bilayer. The dynamics were followed for 5.4 μs.

We also modelled interaction of small Gold, TiO_2 and fullerene NPs with lipid bilayer at the atomistic level and calculated a free energy barrier for penetration, the NP uptake kinetics and free energy of the pore formation. We studied the dependence of the energy barriers for translocation on the bilayer membrane composition, in particular, on the concentration of cholesterol and membrane proteins. We modelled NP insertion into pores in pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine lipid bilayers and those with cholesterol and measured the free energies of pore formation, lifetime of the pores, and possible leakage of the material. No pore recovery was observed within the simulation time.

We analyzed literature data concerned with the toxicity of several groups of widely - spread NPs to describe the modern understanding of the NP - related cytotoxicity mechanisms and the possibility of their assessment via computer simulation: metal oxide particles (TiO_2, ZnO, Fe_2O_3, Fe_3O_4), metal particles (Au, Ag, Ti), carbon based materials (fullerenes, carbon nanotubes, grapheme), polymer particles (polystyrene, polyethylene glycol), quantum dots, semiconductors, binary alloys (CdSe, ZnS).

To derive quantitative criteria for irreparable membrane damage / cell death caused by NP - membrane interaction, we performed an analysis of modern literature in the framework of several problems: evaluation of the membrane damage following the NP - membrane interaction, evaluation of the cell damage following the NP translocation, evaluation of genotoxicity from the NP - Chromatin interaction.

The critical levels of the characteristics that can be related to membrane disfunction were found to be either system specific or not known; nevertheless the desired criteria were derived; they can be used both in experimental and computer simulation studies:

- the total amount of the leaked material: the total mass of various cytoplasmic substances appearing in the extracellular space because of leakage;
- the presence of phosphatidylserine in amounts exceeding certain threshold concentration in the outer leaflet;
- disruption of transport processes through membrane, change of membrane permeability (disturbance of the electronic and/or ion, specific molecules transport activity);
- morphological changes in the system, changes of membrane thickness, bending;
- changes of membrane properties as compared to the initial state such as fluidity, viscosity, elasticity (bilayer elastic modulus), lipid mobility (self-diffusion coefficients of lipids), average values of bond order parameters of fatty acid tails of lipids, etc;
- the characteristic size of pores;
- the characteristic lifetime of pores;
- disturbance of proteins function: protein unfolding or disruption of protein conformation, protein aggregation and/or fibrillation;
- occurrence of genotoxicity / DNA blocking, etc.

We reviewed the available papers concerned with NP cytotoxicity involving intracell mechanisms. The modern understanding of the NP - related cytotoxicity was gained; it involves generating reactive oxygen species, which can increase dramatically, and damage all components of the cell. Two quantitative indicators for NP cytotoxicity involving intracell mechanisms were derived:

- a band gap in metal oxide semiconductor particles (band and hydration energies play roles in the generation of oxidative stress);
- NP solubility.

A scheme of calculation of the NP-associated health risk

We developed a method of describing the state of an organism under various conditions, with the goal of calculation of the NP-associated health risk using a set of measurable nonlethal indicators, i.e., construction of a unified criterion for the risks associated with NP. The common approach contains two stages of several steps each. To obtain a comprehensive description of properties of an organism under the given conditions induced by NPs, a number of nonlethal indicators of diverse nature (experimental and/or theoretical) should be chosen.

At the **first** stage, the measurable indicators should be converted into dimensionless relative values Δ with respect to the controls, in percentage:

$$\Delta_i = \frac{100 \cdot (C_i - C_{i\_ctrl})}{C_{i\_ctrl}}$$

Here Δ_i is the dimensionless relative deviation of the *i*-th indicator from the control value (in percentage); C_i is the *i*-th indicator (e.g., concentration of a substance or an enzyme activity in the given tissue, etc) for the given exposure conditions; C_{i_ctrl} is the corresponding indicator for the control state.

Then the average magnitude of the relative deviation σ_j per indicator over all indicators chosen (characterized state of the organism as a whole under the given conditions) should be calculated:

$$\sigma_j = \frac{\sum_{i=1}^{N_j} |\Delta_i|}{N_j}.$$

Here N_j is the total number of indicators used in the given experiment j, e.g. the experiment with the given organism for the given NPs exposure conditions, $|\Delta_i|$ is the magnitude of the dimensionless relative deviation of the i-th indicator with respect to the control value (in percentage). This approach allows one to combine the indicators from all sources: biochemical studies, physiological, immunological tests, theoretical modeling techniques including computer simulations.

Different conditions of the organism induced by different concentrations of the given NPs or various NPs, if required, can be studied by the same procedure, and corresponding average magnitudes σ_j of the relative deviation per indicator can be calculated for the same set of the indicators. A comparative analysis of the calculated average magnitudes σ_j can be made that allows to reveal the extent to which the organism state is affected by the exposure to NPs.

In the case when the total number N_j of indicators is large enough, the probability density distributions ρ_j of the relative deviation magnitudes of the indicators involved (showing the scatters in their deviations for different conditions) should be calculated.

In the **second** stage, in cases where the range of normal variability (reference range) is known for each indicator, the possibility exists of assessing the organism state as norm or pathology. To assess this state, the normalized relative deviations for all indicators involved should be calculated; weighting factors are firstly calculated for the indicators using their reference ranges, in such a way that the range 0 – 100 % corresponds to the normal variability of the given indicator, and therefore the values over 100 % to pathological response.

The average magnitude of the normalized deviation σ_j^{norm} per indicator over all normalized relative deviations of the indicators involved should be calculated; this parameter allows one to determine the actual state of the organism with respect to the "norm" and "pathology" ranges and thereby to quantify the toxicity level of the considered concentration of the given NPs (and to compare toxicity levels of different NPs, etc). A scheme of calculation of the normalized average dimensionless relative deviation with known reference ranges is presented below:

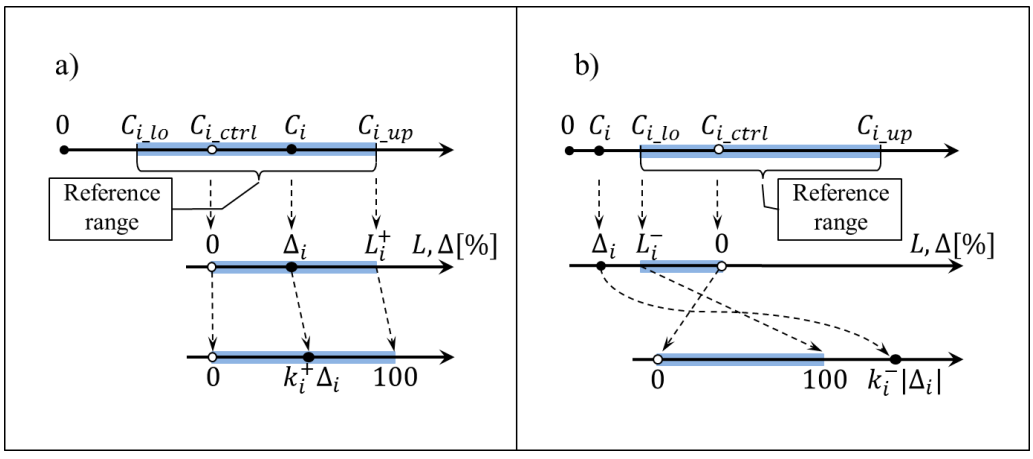


Fig. 18. Scheme of calculation of universal dimensionless health/toxicity indicators. Subfigures a) and b) in this scheme illustrate the calculations for different arrangement of the lower C_{i_lo} and upper C_{i_up} limits of the reference range of the i-th indicator, of its control value C_{i_ctrl}, and the experimental value C_i : a) case $C_i \geq C_{i\_ctrl}$ (and $C_i < C_{i\_up}$); b) case $C_i < C_{i\_ctrl}$ (and $C_i < C_{i\_lo}$).

The scale of "normal" deviations Δ_i (in percentage) of indicator *i* consists of two parts: $|L_i^-|$ and L_i^+. The unified "**normal**" **scale** for *normalized* relative deviations $k_i^+\Delta_i$ or $k_i^-|\Delta_i|$ of each indicator is then mapped onto the standardized range 0 – 100%. Where the *normalized* relative deviations of N_j indicators of the *j*-th experiment are obtained, the *normalized* average dimensionless relative deviation σ_j^{norm} can be calculated. The value σ_j^{norm} = 100% sets the unified limit of normal variability of the set of indicators describing the given state *j* of the organism, so that *proximity* of σ_j^{norm} to 100% already would indicate the risk of toxic effects. σ_j^{norm} can therefore quantify the *degree* of toxicity of the (nano)material, which caused that state.

We tested the methodology using literature data and own data of computer simulation and demonstrated the advantages of the new approach. We were able to compare the toxicity levels and outcomes from different experiments and datasets and draw new conclusions from the published data. The advantages of the suggested approach are that

- it allows one to consider and analyze within the same formalism toxicity-related data obtained from sources of very different nature: *in vivo* or *in vitro* experimental studies and theoretical modeling data including molecular simulations;
- it can be used for quantification of toxic effects of any nanomaterials;
- the indicators used are nonlethal;
- even though an application of only the first stage of the described methodology is possible (in cases where reference ranges are unknown for the indicators), it allows to compare states of an organism under different conditions and to reach toxicity-related results.

The description of the developed toxicity assessment scheme and examples are available through the project website.

Summary for WP5

We developed a method of evaluation of state of health of a living organism based on diverse biochemical and biophysical data. This scheme can also be used for calculation of the NP-associated health risk using a set of measurable nonlethal indicators, i.e., construction of a unified criterion for the risks associated with NP, which can combine the data from any kind of toxicity experiment or simulation. We tested the methodology using literature data and demonstrated the advantages of the new approach.

In addition, we attempted to predict toxicity of several common engineered NPs, using the simulation methods developed in WP2-4. We modelled C_{60} NPs in contact with a DMPC lipid membrane and predicted NP uptake and change of membrane properties upon increase of concentration of inserted NPs. The toxicity indicators were evaluated. We also modelled interaction of small Gold, TiO_2 and fullerene NPs with lipid bilayer at the atomistic level and calculated a free energy barrier for penetration, the NP uptake kinetics and free energy of the pore formation. We studied the dependence of the energy barriers for translocation on the bilayer membrane composition, in particular, on the concentration of cholesterol and membrane proteins.

We modelled NP insertion into pores in pure DPPC lipid bilayers and those with cholesterol and measured the free energies of pore formation, lifetime of the pores, and possible leakage of the material. No pore recovery was observed within the simulation time.

To derive quantitative criteria for irreparable membrane damage / cell death caused by NP - membrane interaction, we reviewed modern literature in the framework of several problems: evaluation of the membrane damage following the NP – membrane interaction, evaluation of the cell damage following the NP translocation, evaluation of genotoxicity from the NP - Chromatin interaction. The critical levels of the characteristics that can be related to membrane disfunction were found to be either system-specific or not known. Nevertheless the desired criteria were derived (they can be used both in experimental and computer simulation studies).

3.7 Conclusions and Outlook

We have successfully completed the methodological parts of the research programme. The main scientific achievement is the development of complete self-consistent framework for modelling NP–cell membrane interaction. It includes the minimal sufficient set of elements of the CG model: NP model, protein model, model for corona formation, model of lipid bilayer, parameters for all the necessary interactions for representative engineered nanomaterials (TiO_2, Gold, SiO_2, CdSe, Carbon). All the components of the framework were validated with experimental data or results of more detailed molecular simulations. With this methodology, we are able to predict (i) content of NP protein corona, (ii) scenarios of the NP-membrane interaction, and (iii) potential degree of toxicity of the nanomaterial based only on its physicochemical characteristics. Moreover, we have identified several weaknesses of the existing computational approaches, if applied to bionano interface, and suggested the solutions, which can serve as a basis for development of smart tools for assessment of nanomaterial toxicity.

4 The potential impact, the main dissemination activities, and exploitation of results

4.1 Projected impacts

MembraneNanoPart project has directly addressed the desired impacts of both the overall NMP Programme, and the specific call "*Modelling toxicity behaviour of engineered nanoparticles*". From a technical viewpoint, our methodology enables the mechanistic modelling of the NPs in a biological environment, and therefore offers a systematic way to identifying the molecular mechanisms of uptake and membrane damage.

The main project outcomes:

- **Techniques to support the development of relationships between NP properties and toxicity (including interactions of nanoparticles with biological systems).** *MembraneNanoPart* has developed theoretical and computational modelling tools enabling a direct insight into the molecular mechanisms of NP interactions with living organisms. *MembraneNanoPart* has created possibilities to characterize the hazards posed by NPs in relation to their ability to cross the protective barrier of the cells, damage the cell membrane, and bind to key biomolecules. Our hierarchical modelling approach (interactions between the particles in a biological environment, interaction with the membrane elements, interaction with cellular membranes, and evaluation of the membrane's final state) provides information on the role of NP-membrane interactions in producing the cytotoxic effects and thereby also provides a useful route for this information to be integrated into predictive approaches.
- **Identification of key physicochemical properties to be chosen for establishing groups of structurally similar NPs, the characterisation and classification techniques, the test methods, and the relation of structural descriptors to toxicological targets**. There are two critically important stages of NP contact with biological environment: at sufficiently small times/fast systemic transport (sub-second to few seconds) the NP preserves its original physicochemical properties and can interact with other particles, biomolecules or the cell membrane by means of its surface groups. At longer times (seconds or minutes after the intake), NPs get coated by a layer of biomolecules that changes their surface properties, with which the NPs will be seen by the cell. We have identified and calculated **new physicochemical descriptors**, which reflect the nanomaterial ability to form complexes

with biomolecules: hydrophobicity and aminoacid binding ability, radius of curvature, Hamaker constants for bulk nanomaterial interaction with proteins and lipids, in addition to the convenient surface properties such as zeta potential. The endpoints that we have chosen to focus on in this programme are interparticle interactions in biofluids (e.g. plasma), interactions with biological membranes (involved in uptake processes), interaction with cells (membrane disruption), and the final state of the cell membranes. These new desctiptors will support the development of new intelligent QSARs relating the NP properties with specific hazards an adverse outcomes.

Research impacts

New development in the computational toxicology

Given that the microscopic processes involving NPs are much different from what has been seen before (for chemicals and pharmaceuticals), our initial steps in modelling of these processes are of significant value for the field. The construction of a general framework began from a case study of only a few particle types, with understanding that the developed methodology can be straightforwardly applied for other particle types since our modelling is based on the physical principles of molecular interactions determined by the quantum and classical mechanics. This is a considerable difference from previous approaches involving knowledge based methods which require enormous amounts of (validated) data, which is not yet present, and which is expected to take some years to build. The project focuses on particular case-study examples to address the key issues – in this case modelling how NPs interact with biological barriers, such as the cell membrane.

Education and training or researchers

All the project PIs were involved in undergraduate and postgraduate teaching activities in the field of nanobiosciences, nanotechnology, materials chemistry, biochemistry, or environmental protection. The advanced modelling approaches, developed within this project, as well as new knowledge on NP-cell interactions and toxicity mechanisms, is now used by the PIs in the postgraduate student training in their respective institutions. Furthermore, three postdoctoral researchers (Dr. Erik Brandt, Dr. Hender Lopez and Dr. Matthew Schneemilch) and one PhD student (Dmitry Zhurkin) were recruited for the project and took an active part in getting experience to work in high-end scientific environment. Dmitry Zhurkin has successfully defended his PhD Thesis in the end of the project. The young researchers were introduced to the best research methods and to Nanosafety cluster research network through consortium meetings and collaborative work, which will help to develop further research links. In addition, in their career they can benefit from the engagement with the leading research groups in the field and acquaintance with the relevant industries dealing with nanotechnology and nanomedicine.

Medium and long term benefits

In the medium term, the project contributes to developing the concept of an inventory of NPs based on categorising NPs on the basis of a determined set of physicochemical properties which can be computed by molecular modelling and linked to the toxicological properties. It enables a mechanism-aware analysis of existing nanotoxicological data by intoducing relevant descriptors for the studied nanomaterials. In this regard, the approach of building in the mechanisms, and representing the specific examples of materials, will in the longer term enable the approach to acquire a predictive capacity. in which a measurement of a set of well defined parameters (such as aminoacids adsorption free energies) will allow for modelling complex outcomes.

The Horizons

The nanotoxicology field is still far from its maturity and faces tremendous challenges. Fundamentally, connecting basic bionano interactions properties of NPs to long-term, chronic adverse outcomes, though highly ambitious, is becoming achievable via combining systems biology studies, which address the complete set of perturbed pathways, with the specific interation scenarios (molecular intitating events or key events). We consider that this will be a foundation step in the development of the field toward the quantitative and reliable risk assessment completely *in silico*, or with limited *in-vitro* tests.

Economic impact

The European Commission has determined that Nanotechnology is one of the key enabling technologies of the future, and both the Commission and the member-states have invested heavily in its success. Thousands of nanomaterials (NM) are already on the consumer market, and in many cases, the risks of personal or environmental exposure to these materials are unknown or poorly understood. Under REACH rules, manufacturers, importers and downstream users are obliged to ensure that the NMs used in their products do not adversely affect human health or the environment yet there is no way of reliably predicting such effects. Concern has been voiced that the safety of NMs has not been addressed satisfactorily. On the economical side, a study of DG Enterprise and Industry has shown that REACH rules may cause administrative burden, affect time to market especially for SMEs.

The *MembraneNanoPart* project provides a systematic framework into which existing and emerging experimental data can be funnelled in order to model the interactions of engineered NPs of particular industrial and economic significance with living systems. We consider that building a rigorous foundation, based on key microscopic physical principles is the most secure chance of being robust. Such a methodology does not behave as a ‘blackbox’, and can be tested and validated at every step, and level of the developmental hierarchy by much more limited experiments and, therefore, less investment (for example, only on the particle dispersions, only on cells, only on barriers, etc).

Relating the physicochemical properties of NPs with their biological fate (uptake and localization in the cell) and behaviour (functional impacts and potential toxicity) will enable categorization of NPs according to physicochemical factors that constitute a “risk” either for bioaccumulation in the cells and tissues or potentially a specific toxicity endpoint. Feeding this information back into the process development will enable manufacturers to quickly screen out particles with physicochemical properties that equate with a risk, and either develop new particles or re-engineer their products to modify their properties, thereby designing out the risk factors initially, and in the longer term potentially designing the NPs to be safe.

As a result it will facilitate the entry of SMEs into the market by removing the cost of outsourcing the screening.

Human impact

While there are significant numbers of products available on the Internet claiming use of nanotechnology, not many have been approved for use in the EU. There are believed to be tens of thousands of NP types under investigation, potentially entering the market. Estimates for the needs of animals for *in vivo* testing exceed what is considered reasonable in any modern society, let alone Europe, where particular emphasis is given towards the need to reduce reliance on animal models. In some arenas (such as cosmetics) particular rules eliminate the potential for animal testing. The *MembraneNanoPart* project has created the methodology which will provide basis of a future screening strategy to predict the likely impact of new NPs.

This is important, for the reliance on a fundamental and mechanistic basis is a much more secure approach to dealing with entirely new particle types. The main scientific results of

MembraneNanoPart provide a new screening strategy, reducing (or removing at all) the need for in-vivo testing, giving reassurance if there are no disease implications identified, whilst providing an opportunity for responsible measures to protect citizens should a nanosafety hazard from engineered NPs emerge.

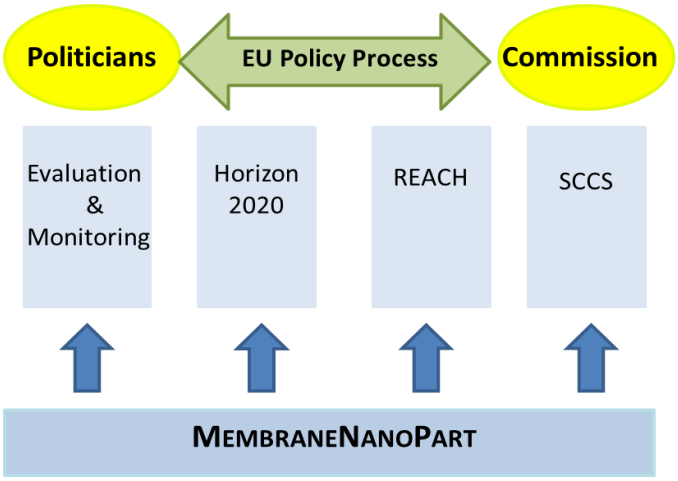
Policy development and regulations

The scientific outputs from the *MembraneNanoPart* consortium is a useful resource for regulatory agencies, and can serve the public need for responsible development of nanotechnology. It will also, as a result of its truly international structure build balanced consensus amongst the principal groups around the world. It will thus make an excellent step along the way to the goals of the "Science and Society –Action plan", COM (2001) 714.

It is clear that as the industry develops, nanotechnologists and regulators have a limited window of opportunity to evaluate the potential risks before products arrive to market. Development of standards and regulations requires for an effective and measured policy response, and with the current lack of data on many NPs emerging modelling tools to take account of the specific features of the nanoscale may become a valuable instrument for regulators.

The *MembraneNanoPart* project has contributed scientifically grounded methodologies to the development of standards, test and measurement methods in the field of NP interactions with biological systems. It has provided essential modelling tools, which will assist in the planning and development of reference technical instruments needed for EU policies such as the revision of Environment Directives, REACH, OECD working group in Nanomaterials, protection of health, food safety, etc.

In the future development, we will be particularly concerned to probe the degree to which these modelling and prediction tools developed within the *MembraneNanoPart* project become relevant to *in vivo* situation and toxicology endpoints, as the optimal situation would be that simple physicochemical characterisation of NPs by molecular modelling tools could predict their fate and behaviour. Building up this support to research at the interface between experimental and modelling efforts will be a major contribution to enhance the impact, and we will seek to apply the same standards across the whole grouping, internationally. We also highlight the fact that the combination of these with high-throughput advanced biological and screening methods could be harnessed in a quantitative manner. The standards and protocols will have valuable impacts in various European and International bodies, such as CEN WG 166, ISO and related bodies. There could also be useful inputs to EU policies such as revision of Environment Directives, REACH, protection of health, food, work, and environmental safety.



4.2 Dissemination activities

During MembraneNanoPart project lifetime we have been very active in disseminating our results. The central actions: four workshops on the topic of bionano interactions, where the members of the consortium were the main organizers, and where all project partners were represented:

- 3rd Zing Conference on Bionanomaterials (6-9 February 2013, Lanzarote, Spain) with over 100 participants
- 4th Zing Conference on Bionanomaterials (6-9 April 2014, Nerja, Spain) with over 100 participants

- CECAM workshop "Molecular and coarse-grained modelling of interactions at bionano interface" (22-24 September 2014, Dublin, Ireland) with over 50 participants

- CompNanoTox 2015 conference with 100 participants (4-6 November 2015, Malaga, Spain)

We participated in EuroNanoForum 2013 (Dublin, Ireland) and 2015 (Riga, Latvia) and presented our research.

We participated in the activities and management of EU FP7 COST action Modena on Modelling Nanomaterial Toxicity.

We participated in Nanotexnology 2015 conference (Thessaloniki, Greece) and presented our research.

NANOTEXNOLOGY 2015
International Conferences & Exhibition on Nanotechnologies & Organic Electronics
4-11 July 2015, Thessaloniki, Greece

We took part in the harmonization meetings organised within modelling group of the Nanosafety cluster and led the bionano interactions Working Group.

In addition to this, the members of the consortium presented 19 talks and 6 posters at international and national conferences.

Industry: the consortium has pro-actively engaged with industry initially via the "European Technology Platform on Nanomedicine and a Dassault Systemes subsidiary Biovia (previously known as Accelrys), and GlaxoSmithKline.

Other contributions to dissemination include 5 journal publications, the project presentations in the Nanosafety Cluster compendium, discussions with eNanoMapper, presentations at the harmonisation meetings, EC materials modelling brochure, updates of the project website http://www.membranenanopart.eu/

4.3 Exploitation plan

One of the main results of *MembraneNanoPart* project is a methodology for efficient modeling of biomolecules, NPs, and interactions at the bionano interface. The main exploitable outcomes are listed below:

Open source software

- Within the work on CG models in WP2, we have further developed **open source package MagiC** and released new versions 2.0 and 2.2 that provided this package with new functionality, which is relevant for development of CG models describing multicomponent lipid bilayers and interactions of lipid bilayers with NPs. Besides the core of the code which performs calculation of the CG potentials, the package contains utilities to transfer atomistic trajectories to CG ones, a user-friendly interface to compute all reference distribution functions, perform post-simulation analysis and to export computed CG potentials to Gromacs format thus enabling their direct use in subsequent mesoscale simulations. The code with all related utilities (gathered in the subpackage MagicTools) and documentation is freely available through group website at Stockholm University, the project website and via open-source repository.
- The implementation of our model on CG model of NP-protein interaction consists of set of scripts for (i) creation of one-bead-per-aminoacid model of protein from PDB files, (ii) making of second CG model (united aminoacid) of proteins for studying competitive adsorption, and (iii) calculation of adsorption energy of arbitrary proteins with known structure onto a NP (Lopez, Lobaskin 2015), which can be used with open source ESPResSo MD package. The scripts can be downloaded from the project website.
- The implementation of our model of NP interaction with membranes consist of set of scripts and input files for Gromacs MD package. We produced scripts for insertion of NPs into pre-equilibrated lipid/water systems. The input files needed to run the simulations of gold NPs interacting with DMPC bilayers using MD package Gromacs 4.5 or later can be downloaded from the project website. Configurations for NPs of size 3, 5 and 10 nm are available with

fully atomistic Slipids bilayers. For CG implicit solvent lipid models input files and configurations with particles of size 10, 15, 20 and 25 nm are also available for download.
- MS Excel spreadsheet for estimation of energy barrier for penetration of NPs through lipid bilayers can be downloaded from project website.

Data: interaction potentials
- Complete set of files needed to reproduce the PMF of a Slipids DMPC molecule with a planar gold surface.
- Effective CG potentials for DMPC, DOPC and DSPC lipids, together with topology files for Gromacs MD package
- United-atom and united-aminoacid models of common plasma proteins for use with ESPResSo MD package
- PMFs for TiO_2 and Gold with amino acids
- PMFs for SiO_2 (quartz and amorphous) with amino acids
- PMFs for CdSe with amino acids
- PMFs for CG lipid fragments with amino acid
- PMFs for interaction of a C_{60} NP with a DMPC lipids and bilayer

The codes and data listed above are available for download on the project website (http://www.membranenanopart.eu/outcomes.php).

The codes and methods developed within the project have potential commercial value. We envision that the results of this project can be exploited for the benefit of the EU in at least three ways:

1. Through our advisors Biovia we will look to transfer the advances in NP/membrane modelling made during *MembraneNanoPart* to academics and industrial users. In particular, we envision the following methods/software elements to be exploitable
- improved force fields for inorganic nanomaterials
- coarse-graining methodology for NP / lipid interactions
- coarse-graining methodology for NP / protein interactions
- multiscale methodologies from atomistic through to CG
- toxicity assessment methods.

We will start with sharing the software codes with the research community via open source repositories (GitHub). A community validation and experience will form the basis for further improvement of the methods and their exploitation in successor projects. This will lead eventually to a commercial nanotoxicity prediction code and its introduction to EU industry though industrial consortia led by Biovia, who will be a formal partner in the successor projects. Biovia has been in the forefront of the development of QSAR tools and has many in-house experts. In particular, Biovia has previously developed tools (ADME/TOX) to predict the toxicity of small organic molecules that are currently used in the industry.

2. The results will be used in the current EU FP7 (eNanoMapper) and upcoming EU H2020 projects (SmartNanoTox) for producing bionano interactions database and development of smart secreening tools.

3. The computational methodology will be shared with the materials modelling community via our participation in the European Materials Modelling Concil (EMMC). We will adopt the community-approved ontologies and data exchenge format to facilitate the use of the code by industrial stakeholders.

4. The results of this project will be used in training courses in the partner institutions as well as relevant nano-training courses in partner and other EU countries and video-lectures will be available at project and partner websites.

Commercialization of computational tools

From *MembraneNanoPart* simulation methods, several modelling tools will be created. Upon community testing and validation, these will adapted and finally inserted into commercial (e.g. Biovia) molecular modelling software for commercial exploitation. In particular, atomistic and mesoscopic *builders* is a very important enabling technology for Biovia customers and save potentially much research time. They can make the difference between success or otherwise, and differentiate Biovia from many competitors. Specific nanoparticle builder could thus be implemented in the Materials Studio® suite of software and distributed using current commercial channels. Technology available to be transferred includes (short term) methods for coarse-graining potentials to include solvent (water), methods for developing CG potentials for nanoparticles, and methods of toxicity assessment, longer term CG methodologies for lipid bilayers with NP wrapping will be offered to Biovia.

Training Courses

Methods	Course	Institution/data
Membrane models	MRES nanomaterials	Imperial College, February each year
NP-membrane and protein interaction models	Nanomechanics, MSc Nano bio Physics	University College Dublin, September-November each year
Coarse-graining techniques for biomolecules and colloids	Modelling in Materials Chemistry, MSc Materials Chemistry	Stockholm University, February-March each year.

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