Chapter 10
Compilation of Data and Modelling of Nanoparticle Interactions and Toxicity in the NanoPUZZLES Project

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Abstract The particular properties of nanomaterials have led to their rapidly increasing use in diverse fields of application. However, safety assessment is not keeping pace and there are still gaps in the understanding of their hazards. Computational models predicting nanotoxicity, such as (quantitative) structure-activity relationships ((Q)SARs), can contribute to safety evaluation, in line with general efforts to apply alternative methods in chemical risk assessment. Their development is highly dependent on the availability of reliable and high quality experimental data, both regarding the compounds' properties as well as the measured toxic effects. In particular, "nano-QSARs" should take the nano-specific characteristics into account. The information compiled needs to be well organized, quality con-

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trolled and standardized. Integrating the data in an overarching, structured data collection aims to (a) organize the data in a way to support modelling, (b) make (meta) data necessary for modelling available, and (c) add value by making a comparison between data from different sources possible.

Based on the available data, specific descriptors can be derived to parameterize the nanomaterial-specific structure and physico-chemical properties appropriately. Furthermore, the interactions between nanoparticles and biological systems as well as small molecules, which can lead to modifications of the structure of the active nanoparticles, need to be described and taken into account in the development of models to predict the biological activity and toxicity of nanoparticles. The EU NanoPUZZLES project was part of a global cooperative effort to advance data availability and modelling approaches supporting the characterization and evaluation of nanomaterials.

Keywords Nanoparticle • Nanomaterial • Toxicity • Interactions • Data compilation • Data quality • Data standardization • Nano-descriptors • Nano-QSAR

10.1 Introduction

The number of nanomaterials and their applications in nanotechnology is increasing rapidly and will grow considerably in the foreseeable future. It is the particular properties of the nanomaterials, based on specific characteristics such as the particle shape and size distribution, surface area and chemistry, solubility and porosity as well as the state of agglomeration and dispersion, which have led to their increased use in diverse fields of application such as medicine, cosmetics, food and textile products, water treatment, catalysis or in construction and electronics.

However, safety assessment of nanoparticles is not keeping pace with their rapid development and there are still gaps in the understanding of the hazards posed by nanomaterials. The basic requirement for establishing a nanoparticle-specific hazard assessment is the availability of reliable and consistent nanotoxicity data, associated with high quality characterization of the identity and structural/physico-chemical properties of the materials investigated. There are many efforts worldwide to elucidate mechanisms of action for nanoparticles and generate toxicity data for diverse endpoints. However, the publication of the data is disperse and thus makes their comparability and uptake for safety assessment difficult.

Risk assessment of all chemicals including nanoparticles is increasingly being supported by computational methods, such as (quantitative) structure–activity

relationships ((Q)SARs), based on the understanding that the biological activity of a substance is related to its chemical structure and physico-chemical properties. The development of these in silico approaches is highly dependent on the availability of reliable and high quality experimental data. QSAR models are well established for different endpoints for organic molecules, however, the same techniques cannot necessarily be applied directly to nanoparticles. As such, so called "nano-QSARs" have to take the nano-specific characteristics of nanoparticles into account. Therefore the compilation of data of high quality, both regarding the compound characteristics, particular for nanomaterials, as well as the measured toxic effects, is imperative as the basis for the modelling of nanotoxicity. Ideally these data should be made available in a unified form and in an open, electronic, easily accessible format.

Within the 3-year European NanoPUZZLES project (European Commission 7th Framework Programme, FP7-NMP-2012-SMALL-6, grant agreement no. 309837), from January 2013 to December 2015, the partners University of Gdańsk (Gdańsk, Poland), Istituto di Ricerche Farmacologiche Mario Negri (Milan, Italy), National Hellenic Research Foundation (Athens, Greece), Liverpool John Moores University (Liverpool, England) and BioBaltica (Gdańsk, Poland) have collaborated to advance knowledge and computational methods for modelling the relationships between the structure, properties, molecular interactions and toxicity of nanoparticles. The work has been organised into four complementary thematic areas (the "puzzles"), namely: NanoDATA, collation and evaluation of available physico-chemical and toxicological data for nanoparticles; NanoDESC, descriptors specific to nanoparticles and their characteristic features; NanoINTER, interactions of nanoparticles with biological systems; NanoQSAR, using the knowledge gained from within the first three puzzles to understand the relationships between experimental toxicity data and nanoparticle properties and develop nano-QSAR models. The project has focussed on two groups of nanomaterials: inorganic nanoparticles such as metal oxides and carbon nanomaterials such as fullerenes and fullerene derivatives.

A general approach to data driven modelling of nanomaterial toxicity, starting from the review of available data, is summarized in Fig. 10.1 [25]. The data collection entails consideration of the data quality and suitability of the data for modelling (e.g. availability of key endpoint data for a suitably large number of nanomaterials obtained from a single source) as well as standardization of the format and terminology, essential for nanotoxicity modelling, also in view of the broader application of the data by integration into overarching databases, supporting predictive model development and safety evaluation of nanomaterials.

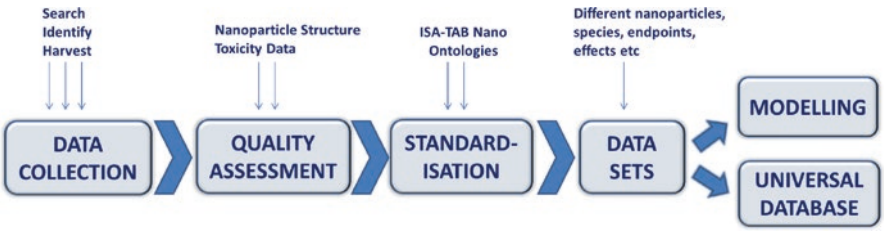

Fig. 10.1 A general approach to data collection to model nanoparticle toxicity

10.2 Data Collection

Reliable information on the structural characteristics and physico-chemical properties of nanomaterials, as well as available experimental data on biological effects, is the basis for the development of nano-QSARs. Therefore, the first step for modelling is to identify and collate available data, from the scientific literature or, for example, other research initiatives generating data on nanomaterials.

Compiling the data in an overarching, even inter-project or global, data collection has the following aims:

(a) to organize the data in a form to optimally support the modelling,
(b) to ensure all (meta)data necessary for modelling are available,
(c) to add value to the available data by making comparison of data from different sources possible and thus to enable the integration of these data from diverse efforts to characterize nanomaterial hazard for the development of models to support nanosafety assessment.

To achieve these aims, particular efforts have been made in the NanoPUZZLES project to identify available data through a comprehensive literature review and these data were evaluated according to the requirements for building a data collection useful for nano-QSAR modelling. In order to merge heterogeneous datasets and make them comparable, a suitable standardised data format and vocabulary is needed.

Within the NanoPUZZLES project, data from more than 300 publications were identified, including data obtained through the NanoBridges project (FP7-PEOPLE-2011-IRSES, grant agreement no. 295128), principally focussing on the endpoints cytotoxicity, genotoxicity and aquatic toxicity for fullerenes, carbon nanotubes, metal/metal oxides and silica nanoparticles. Physico-chemical and structural information which were identified also included size characterisation measurements from multiple techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS).

The specific procedures and suggestions for the standardisation and quality assessment of the information collected are described below.

10.2.1 Data Quality and Availability

The quality of an in silico prediction model depends on the quality of the data used for the model development. Therefore the consideration and evaluation of the data quality is a crucial step for modelling.

Data suitability considerations comprise three different areas, which include evaluation of some nanomaterial specific properties, as follows:

- the amount of data and their quality sufficient or necessary for modelling
- the quality and completeness of the nanomaterial structural characterization and physico-chemical properties data,
- the quality of the nanomaterial toxicity data.

Lubinski et al. [14] suggested that at least 10 data points, from a single source, are required for (Q)SAR modelling. A literature survey was performed in NanoPUZZLES and the results confirmed that it is a challenge, in practice, to achieve this criterion. Out of a sample of 363 primary experimental articles considered in 2014, with cytotoxicity and genotoxicity data for nanomaterials, most reported on too few nanomaterials/data points to develop models based on data from a single source. The use of data from different sources would improve the data availability situation, if they were sufficiently comparable. Therefore the recording of (physico-chemical and toxicity) data in a standardized form is the prerequisite to compare data from different sources and possibly allow the development of models based on multiple sources. The standardization of data recording is discussed in the next section.

Lubinski et al. [14] proposed a general set of quality criteria for any kind of nanomaterial experimental data, based on Klimisch scores [9]. The Nanomaterial Registry proposes a set of "compliance levels" [18, 20] for evaluating the curated physico-chemical data. These approaches were considered when developing proposals for data quality and completeness assessment within NanoPUZZLES. In addition, published proposals regarding priority physico-chemical characterization parameters [31, 32] as well as the need to consider the potential for artefacts and misinterpretations in data obtained from nanotoxicology tests [21] was taken into account to develop provisional data quality/completeness schemes for assessing the curated physico-chemical and toxicity data.

The importance of transparency of this evaluation has to be emphasized, i.e., as also recommended for the ToxRTool [29], all outcomes obtained from evaluation of individual checklist criteria as well as the final quality assessment should be reported to allow for critical evaluation of the quality assessment by end-users. The most appropriate manner in which the completeness and quality of curated nanomaterial data should be evaluated remains a critical question (see Marchese Robinson et al. [16]).

10.2.2 Standardization of Data Recording

To make it possible to integrate data(sets) from different sources and experimental measurements into one database, there is a need for standardization of the data format, the vocabulary and metadata used. This also provides the necessary basis to allow for the possible comparison of heterogeneous experimental data.

ISA-TAB-Nano [6, 15, 34], an extension of the ISA-TAB specification for reporting of data from biological experiments based upon a standardized representation of (meta)data [26, 27], was proposed as a global standard for nanomaterial data sharing and has been adopted as such for the nanotoxicity modelling projects. Specific data collection templates, designed to support collection of specific nanosafety data and metadata, were developed within NanoPUZZLES [15]. Columns and rows were pre-defined based upon relevant items of (meta)data capturing key physico-chemical measurements, toxicological data and experimental conditions.

The ISA-TAB-Nano specification is based upon a set of four linked spreadsheet-like tab-delimited text file types, designed to capture different kinds of nanoaterial (meta)data from experimental measurements, the Investigation, Study, Assay and Material files, with a pre-defined file structure and syntax for (meta)data. The Study and Assay files describe the samples and the corresponding assays respectively, including assay conditions and measured values. A Material file records chemical composition information, associated with the original nanomaterial sample, and (as of ISA-TAB-Nano version 1.2) nominal/vendor supplied characteristics. There are two types of studies: "physico-chemical characterization" or "*in vitro* and *in vivo* characterization" studies. For the former, the (derivative) nanomaterial sample is considered "the sample" which is being tested in an assay whilst, for the latter, the (derivative sample of the) biospecimen is considered "the sample" and the nanomaterial is considered an experimental variable whose effect on the assay outcome is being evaluated – or, in the nomenclature of the ISA-TAB-Nano specification, a "factor". The Investigation file links all of the previous three file types together and allows for links to additional metadata, such as primary literature references and links to ontologies. Figure 10.2 shows the concept of the links between different ISA-TAB-Nano file types schematically.

These file types are named according to the following conventions: "i_xxxx.txt" (Investigation files), "s_xxxx.txt" (Study files), "a_xxxx.txt" (Assay files), "m_xxxx.txt" (Material files) where "xxxx" denotes some unique file identifier. Figure 10.3 shows a simplified example of how the linked ISA-TAB-Nano files were used to record data from a literature source in the NanoPUZZLES project. In addition, ISA-TAB-Nano also specifies how to create links between nanomaterial samples and any kind of external (raw) data files as well as images, e.g. from transmission electron microscopy.

In addition to the standardization of the "technical" data format, it is essential to define a) a consistent vocabulary to describe the information and, b) which information on the nanomaterial characterization and toxicity assays needs to be recorded

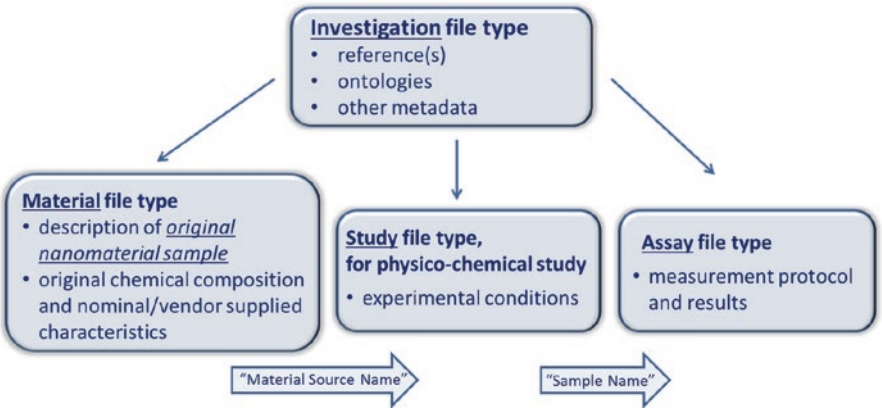

Fig. 10.2 ISA-TAB-Nano defines a set of linked spreadsheet files (Investigation, Material, Study, and Assay), with a pre-defined file structure and syntax for (meta)data. Capture of physico-chemical (meta)data is illustrated

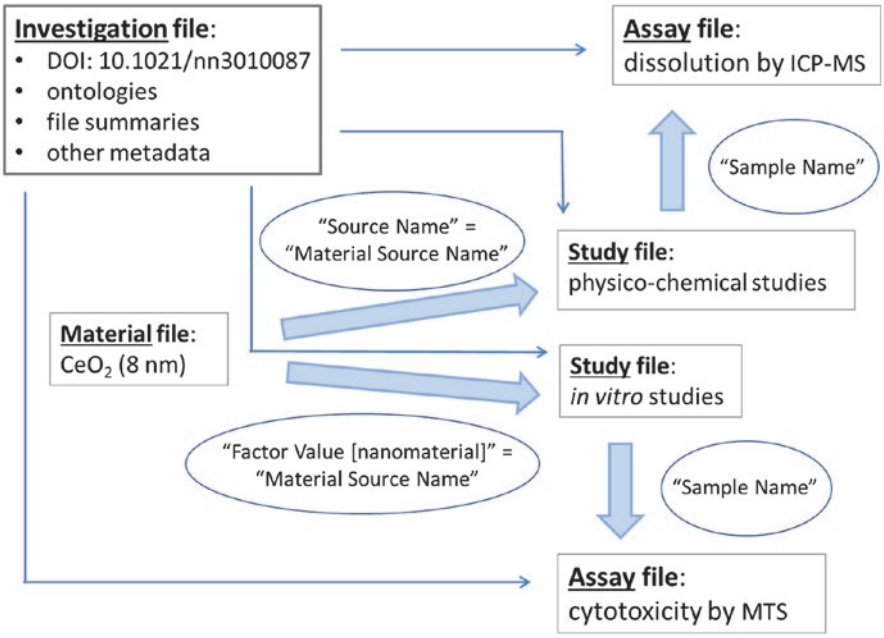

Fig. 10.3 A simplified illustration of how linked ISA-TAB-Nano files are used to record data derived from physico-chemical characterization as well as biological characterization assessments of nanomaterials reported in a single primary literature reference (Adapted from Marchese Robinson et al. [16]. Creative Commons Attribution License, http://creativecommons.org/licenses/by/2.0)

to ensure possible comparison and valid deduction of models (minimum information requirements). While the generic ISA-TAB-Nano specification pre-defines file structure and syntax, it does not specify all (meta)data to be recorded.

Recording data in a unified, consistent terminology is required to avoid inconsistencies, for example due to synonyms. Therefore, for data extracted from the literature, links to terms from ontologies, retrieved using BioPortal [44], for example the NanoParticle Ontology [33], were identified. Links to those ontologies are established via the Investigation file. The selection of appropriate terms is still a challenge due to inconsistent definitions of terms and incompleteness of existing ontologies. This standardized labeling facilitates the possibility to filter the compiled data collection, for example to find all data associated with the same specific toxic effect, and allows comparison of data measured in different experiments.

The adequate characterization of the nanomaterial and description of the assay performed are crucial to allow exploration of intra-and inter-assay and -laboratory comparison and thus to clearly define a uniform basis for the development of nano-QSAR models. For example, an adequate description of the structure is needed to characterize a nanomaterial. A comprehensive and universal format for uniquely representing nanomaterial structures, however, is not yet established, due to the complex nature of nanomaterials. Therefore chemical structures of the nanomaterials were recorded where possible, e.g. for fullerenes and their derivatives, using the SMILES (Simplified Molecular-Input Line-Entry System) line notation. However, the inherent complexity of the nanomaterial structures makes this notation inapplicable for most nanoparticles. In some cases, partial representations based on Crystallographic Information Files (CIF) are available. The ISA-TAB-Nano Material and Assay files are used to record key structural/physico-chemical data such as crystal phase and size information or link to transmission electron microscopy image files.

In general, to ensure a certain quality standard in terms of completeness, minimum information requirements need to be determined regarding which (meta)data should be recorded, i.e. to make certain that all necessary information for modelling the biological activity/hazard of nanomaterials are made available and to allow the evaluation of whether measurements were obtained under sufficiently similar experimental conditions to allow the combination in one single modelling dataset. Proposals for nanomaterial data Minimum Information Standards have been discussed in the literature, with an emphasis on the parameters required to adequately define the structural and physico-chemical nanomaterial properties, e.g. by the Minimum Information on Nanoparticle Characterization (MINChar) initiative [16, 31, 32].

The ISA-TAB-Nano specification, as proposed by its developers, does not specify exactly which nanomaterial characteristics, experimental details and measurements should be recorded. Therefore extended data collection templates were designed within NanoPUZZLES to capture important toxicity and physico-chemical/structural measurements and key experimental details [15]. Within NanoPUZZLES it was suggested that the minimum information criteria should sufficiently define the exact parameters to be recorded. For example, the list of parameters proposed by the MINChar initiative [32] to be reported for nanoparticles in toxicological studies only states “particle size/size distribution” as an essential

parameter. It would be useful to further specify how to record this, e.g. as the z-average hydrodynamic diameter and polydispersity index determined from dynamic light scattering.

10.2.3 Contributions to a Universal Database

The physico-chemical and toxicity data of nanomaterials are collected with the aim of supporting the modelling of nanomaterial properties and toxicity as well as to build an increasing data basis to support hazard assessment of nanomaterials. Ideally all data should be integrated in one universal database, or be searchable through interoperable databases.

The assessment of the data quality and adherence to minimum information requirements as well as their preparation in a standardized format contributes to the effort of organizing the diverse experimental data for inclusion in a database. The aim of the data collection, quality evaluation and standardization is to organize the data and to also allow for a comparison between data from different sources and thus give added value to the data collection. This possible comparison of different data collected for the same nanomaterials will bring together the dispersed efforts to characterize nanomaterials to support safety assessment. Therefore the characterization of the sample and adequate description of the experimental measurements are crucial to allow intra-and inter-assay and -laboratory comparison.

For this overall aim, the general intention is to feed the collected, evaluated and standardized data into an interoperable, searchable nanotoxicity database, which may be consulted on its own to identify hazards and support nanomaterial risk assessment, or used as the data basis for the development of predictive models. The preparation of datasets based on the ISA-TAB-Nano specification was done in view of integration of these data within external databases. For example, NanoPUZZLES ISA-TAB-Nano datasets were submitted to the nanoDMS online database, developed within the EU MODERN project [19].

10.3 Modelling Interactions and Toxicity of Nanoparticles

In silico models such as (quantitative) structure-activity relationships ((Q)SARs), are developed from experimental data and are based on the premise that the biological activity of a substance, e.g. an observed effect on human health or environmental species, can be related to the chemical structure and physico-chemical properties of the compound. This relationship can be expressed as mathematical equations including descriptors of the relevant physico-chemical properties.

Although traditional QSARs have been successfully applied to nanomaterials in some cases, more specific approaches are needed for the model development for nanoparticles [45].

10.3.1 Need for Specific Nano-descriptors

In order to develop QSARs for nanoparticles, suitable descriptors need to be found taking into account the specific characteristics of nanoparticles and their relationship to the activities to be modeled, i.e. in particular to describe the structure and nanoparticle physico-chemical properties appropriately [3, 5, 23].

The development of novel descriptors for nanoparticle structures was the aim of the NanoPUZZLES NanoDESC work package. The "nano-descriptors" developed should reflect the essential properties of nanomaterials such as parameters characterizing the particular nanoparticle structure and electronic states resulting from quantum effects of the nano-size. An overview of the type of the general characteristics described by 0D to 4D descriptors and examples of specific nanoparticle properties are given in Table 10.1.

While the chemical structure of a substance can be generally represented by, e.g., an orbital graph, a 2D SMILES string or the unique InChI identifier, a novel concept is needed for the majority of nanomaterials to express the specific features of the "nano structure": The molecular architecture of nanoparticles is very large and complex, with inorganic and organic elements and exact stoichiometry varying between materials [2], and it is not always possible to represent the specific interactions between different parts of the nanosystems topologically and/or by means of molecular and quantum mechanics.

A possible approach for the creation of a model for nanomaterial(s), when traditional descriptors such as 2D topological indexes, 3D stereochemical and quantum mechanical descriptors are not adequate, is to consider the measured endpoint as a mathematical function of all available eclectic information. The traditional QSAR paradigm 'Endpoint = *F*(molecular structure)' will therefore be replaced with

Table 10.1 Molecular descriptors: simple mathematical representation of a molecule, used to encode significant features of molecules

Dimensions	Characterization
0D descriptors	Constitutional
1D descriptors	Physico-chemical
2D descriptors	Topology of a molecule
3D descriptors	Based on a space representation of a molecule
4D descriptors	Various conformers of the same compound

Properties	Experimental measurements
Shape and aggregation	High resolution microscopy
Composition, purity and surface chemistry	Spectroscopy and chromatography methods
Surface charge	Zeta potential analysis
Crystal structure	X-ray diffraction
Particle number and size distribution	e.g. SEM, TEM, DLS
Surface area	Brunauer-Emmett-Teller adsorption

SEM scanning electron microscopy, *TEM* transmission electron microscopy, *DLS* dynamic light scattering

'Endpoint = *F*(eclectic information)' for nano-QSARs [40]. This information includes atomic composition, conditions of synthesis or preparation of the nanomaterial, as well parameters such as particle size and its distribution, agglomeration state, porosity, particle shape, symmetry, surface area and charge, coating, metal content, dissolution, crystal structure, electronic properties (reactivity, conductivity, interaction energies), chiral vectors of nanotubes, number of walls in the nanotubes [4]. Table 10.2 shows examples of different descriptors applied to the modelling of nanomaterials to predict the listed properties.

It should be noted that (most of) the nanospecific properties cannot be calculated but have to be provided experimentally for the specific nanomaterial considered (see Table 10.1). In consequence the nano-QSAR models are dependent on experimental measurements, rather than calculated descriptors from the molecule representation, and the experimental procedure to determine the respective values may influence the model quality. Moreover, the characterization of the applicability domain of a nano-QSAR model has to be extended consequently according to the new types of descriptors (see for example [41]).

Further examples of descriptors include the metal electronegativity (χ), the charge of the metal cation corresponding to a given oxide (χox), the atomic number and valence electron number of the metal, which were used as simple molecular periodic table-based descriptors for QSAR model development, for example to predict the cytotoxicity of metal oxide nanoparticles towards *E. coli* [7].

In collaboration with the NanoBridges project, scanning electron microscope (SEM) and transmission electron microscope (TEM) images of nanoparticles were computationally processed and analyzed by means of a customized algorithm implemented in the ImageJ software to obtain new descriptors. Morphological (large scale) features were analyzed in relation to shape and size and expressed in the form of ten key descriptors: area, perimeter, major axis, minor axis, aspect ratio, maximum Feret's diameter, minimum Feret's diameter, roundness, circularity, and solidity.

The "Liquid Drop" Model (LDM) descriptors were also developed with the NanoBridges project. They describe the geometric and volume features of studied metal oxide nanoparticles. The model is based on the representation of the nanoparticle as a spherical drop, the elementary particles are densely packed and the density is equal to the density of bulk [30].

10.3.2 Modelling Interactions of Nanoparticles with Biological Systems and Small Molecules

The knowledge of the interactions between nanoparticles and biological systems, such as DNA, proteins and membranes is limited. However, these interactions can lead to the formation of protein coronas, particle wrapping, intercellular uptake and biocatalytic processes, and thus modify the structure of the active nanoparticle, which has an impact on model development, e.g., the descriptors to be calculated.

Table 10.2 Examples of descriptors used to predict nanoparticle properties

Nanomaterials	Descriptors	Predicted property	Reference
24 metaloxides	Conduction band energy levels	Ability to induce oxygen radicals, oxidative stress, and inflammation (cellular redox potential)	[47]
17 metaloxides	$\Delta H_{Me+,}$ representing the enthalpy of formation of a gaseous cation having the same oxidation state as that in the MOx structure	Cytotoxicity (EC_{50}: the effective concentration of a compound resulting in a 50 % reduction in bacteria viability)	[24]
29 (metaloxides, nitrites, silicon carbide)	Product of the correlation weight (DCW) DCW = ΠIkCW(Ik)	Prediction of Young's modulus	[35]
Fullerene C_{60}	RNCG-relative negative charge (Zefirov's PC), ASIC-average structural information content, Eminee(C-C)-min: exchange energy for the C-C bound	Solubility in n-heptane	[17]
Fullerene C_{60}	Correlation of Highest Occupied Molecular Orbital (HOMO), certain heteroatom fragments, and geometrical parameters with solubility	Solubility in organic solvents	[22]
Carbon nanotubes	Chiral vector components (the components of chiral vector contain information about rolling up graphite layer in formation of carbon nanotubes)	Water solubility (log S, S in mol/L) and octanol water partition coefficient (log P)	[37]
Carbon nanotubes, r-graphene oxide, Ag-silica, Ag-carbon, C_{70}TGA, $nC_{60}(OH)_{20}$, $nC_{60}(OH)_{32}$, SiO_2, TiO_2)	Hydrophobicity, hydrogen bond, polarity/polarizability, and lone-pair electrons	Surface adsorption forces (the nanomaterial interaction with biological components)	[46]
Carbon nanotubes	C(N2)/C(T) (number of non-sp^2 hybridized carbons per total carbons) and chiral angle identified as critical descriptors for both Young's modulus and Poisson's ratio	Mechanical property prediction (for Young's modulus and Poisson's ratio)	[1]

Therefore, the aim of the NanoINTER part of the NanoPUZZLES project was to develop methods to simulate and predict interactions between engineered nanoparticles and biomolecules, their environment (e.g., solvents) as well as small molecules.

For the development of a computational protocol for the reliable calculation of the properties of large interacting systems, extensive calculations have been performed, which involved: a large array of techniques (e.g., semi-empirical-PM6-, HF, DFT-B97D, wB97XD, M062X, B3LYP, PBE0-, MP2, molecular dynamics, molecular mechanics Poisson-Boltzmann surface area (MM-PBSA)), and basis sets (e.g., 6-31G*, 6–31+G*, 6-311G*); ab initio techniques for the calculation of the intermolecular interaction energy (e.g., the Su and Li approach, Kitaura and Morokuma (KM) method, effective fragment potential approach (EFP), a variational perturbation scheme); the atoms-in-molecules (AIM) technique for the computation of the hydrogen bond energy; geometry optimization techniques (e.g., B97D/6-31G*, PM6). Several properties, such as the interaction energy, the energies of the Highest Occupied Molecular Orbital (E_{HOMO}) and Lowest Unoccupied Molecular Orbital (E_{LUMO}), ionization potential, electron affinity, dipole moment, polarizabilities and first hyperpolarizabilities, binding free energies, hydrogen bond energies, and structural features were computed. A variety of nanoparticles were used to evaluate their structure and interaction properties, for example: $C_{24}H_{12}$, $C_{84}H_{20}$, $C_{24}H_{12}$, $C_{114}H_{30}$, $C_{222}H_{42}$, $C_{366}H_{54}$; C_{60}, $C_{60}F_n$, $(TiO_2)_n$, single wall carbon nanotube (SWCNT, C_{360}). The calculations were tested on a variety of biomolecules, such as proteins or protein fragments (e.g., HIV-1PR, renin, a G protein-coupled receptor (GPCR), human serum albumin, and human DNA topoisomerase II-alpha).

In conclusion, the following computational methods were recommended:

- A DFT technique for computing the interactions of nanoparticle-nanoparticle systems.
- Ab initio techniques for calculating the interaction properties of biomolecule-biomolecule systems: the AIM method for the computation of the hydrogen bond energy and the Su and Li method for the calculation of the interaction energy.
- Molecular dynamics for studying large biomolecule-nanoparticle or biomolecule-biomolecule systems: the AIM method for the computation of the hydrogen bond energy and MM-PBSA for the calculation of the interaction energy.

The interaction energy of a series of fullerene derivatives with human serum albumin (HSA), or appropriately defined models of HSA, has been computed and resolved into a variety of meaningful contributions by employing ab initio methods. MM-PBSA for the resolution of the binding free energy has also been used. After a detailed analysis of the large body of the computed data, the resolution of the interaction energy yielded the following results:

- For small or average size systems, the use of the Su and Li method in connection with DFT may provide valuable information on the interaction mechanism.
- The fragment molecular orbital method gives very useful information for the whole interacting system, but it is computationally demanding.

- For large interacting systems, the employment of MM-PBSA is recommended.

Furthermore, the interaction with the solvent environment was investigated. Water soluble fullerene derivatives are essential for many biomedical applications. Several studies have examined the relation between solubility and toxicity [28]. Pilot computations on the solubility of several fullerenes were undertaken. It was shown that the solubilization energy (ΔE) depends on the dielectric constant (ε) and the functional groups attached to the C_{60} core, while an increase in ε leads to an increase in $|\Delta E|$. Also, it was shown that the solvent may have a significant effect on some properties (e.g., the average polarizability) of the interacting system.

Three different geometry conformations were used to study the interaction between a fullerene derivative and a model of graphene oxide, i.e. between two different nanoparticles, in aqueous solution. The observed energy difference between the two dominant conformations may be attributed to the number of hydrogen bonds formed between the fullerene and the surface of graphene oxide.

Additionally, molecular aggregation of fullerene systems is obtained spontaneously in aqueous solution, as represented by stable hydrophobic clusters throughout the molecular dynamics simulations. Conversely, water-soluble fullerenes do not form agglomerates in water regardless of their concentration and initial member separation. Moreover, to study the relationship between aggregation and toxicity, (e.g., [13]), pilot computations were performed on the aggregation of fullerenes, (C_{60})n, n = 1–6, where it was shown that:

- Aggregation has a negligible effect on the considered properties.
- The solvent appears to have a minor effect on the properties of (C60)n.

Another objective was to connect the genotoxicity/mutagenicity of a series of fullerenes, $C_{60}F_n$, n = 10, 12, 14, 16 and 18, with their solubility. It is known that E_{HOMO} increases with mutagenicity. This knowledge allowed for the relationship between mutagenicity and solubility of $C_{60}F_n$ to be investigated since it was shown that an increase in n was followed by an increase in solubility and a decrease in mutagenicity. It was also demonstrated that mutagenicity increases with lipophilicity (logP), Q_F (the sum of the charges on the fluorine atoms of $C_{60}F_n$), E(S-T), the dipole moment, the average polarizability, and the first hyperpolarizability. Therefore, a relationship connecting mutagenicity with solubility and a set of important electronic descriptors may be established. The interaction of $C_{60}F_n$ with DNA and human DNA topoisomerase II-alpha (HT2a) was also studied by performing molecular dynamics and MM-PBSA free energy calculations. Significant interactions have been found between $C_{60}F_n$ and both systems, particularly between $C_{60}F_n$ and HT2a. These interactions may induce an undesirable effect on the DNA function.

Finally, the effect of: (i) chemical composition, (ii) size and shape, (iii) aggregation, (iv) surface charge, and (v) contamination of nanoparticles upon a series of properties, such as the structure, the binding free energy (ΔE_{bind}), the interaction energy (E_{int}), E_{HOMO} and E_{LUMO} of several nanoparticle-biomolecule systems was investigated. The nanoparticles investigated were: a) molecular graphene ($C_{84}H_{24}$,

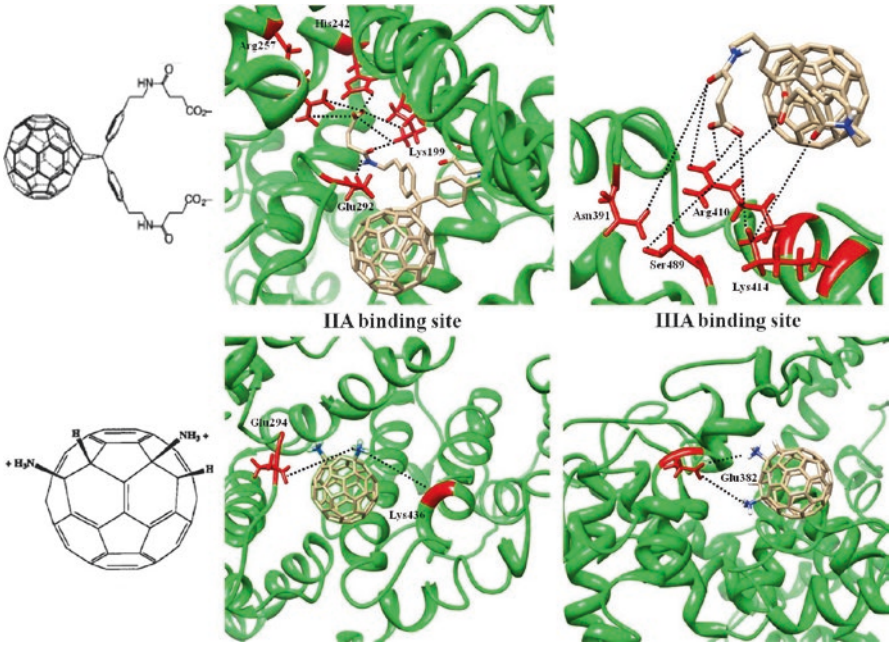

Fig. 10.4 Hydrogen bond interactions between fullerenes and human serum albumin. Main hydrogen bonds between fullerene derivatives and the binding cavities of human serum albumin are shown as dotted lines. Residues that are involved in interactions with the compounds are highlighted (Adapted with permission from Leonis et al. [13], Copyright (2014) American Chemical Society)

$C_{60}B_{12}N_{12}H_{24}$, $C_{78}H_{24}FeN_4$), b) C_{60} and $(C_{60})_4$, c) two single wall carbon nanotubes ($C_{360}H_{40}$, $C_{180}H_{20}$), d) a series of functionalized C_{60} derivatives, and e) $C_{60}F_n$, n = 10, 12, 14, 16, and 18. For biomolecules, the following systems were used: a) the five nucleobases (NBs), thymine (T), cytosine (C), guanine (G), adenine (A) and uracil (U), b) the guanine tetramer (G4), c) HSA, d) HT2a, and e) a DNA sequence were considered. The ab initio study on the effect of size and shape of the nanoparticle was performed on complexes: $C_{84}H_{24}$-X/Y, C_{60}-X, $C_{360}H_{40}$-X/Y, $C_{180}H_{20}$-X, where X=guanine and Y=adenine. It was found that E_{int} is mostly affected by the size and shape and in a lesser extent by $E_{HOMO/LUMO}$. Molecular dynamics runs for fullerenes bound to HSA showed that compounds with longer groups attached to the fullerene core strengthen HSA binding, while compounds with shorter groups diminish protein binding (Fig. 10.4). For the study of the aggregation effect, the $(C_{60})_4$-G4 complex was used. A significant change of the G4 geometry was observed and a high E_{int} (−18.4 kcal/mol) was computed. The effect of the surface charge was studied by computing the E_{int} of C_{60}Q-G, where $Q = 0, -2, -4$. It was found that the charge of C_{60} has a remarkable impact on E_{int} and on the geometry of guanine. Regarding HSA complexes, the calculations showed that negatively charged groups on the fullerenes

are necessary to produce profound binding effects [12]. Also, fullerene binding to the IIA site of HSA was associated with allosteric modulation of IIIA and heme binding sites. The above findings may be particularly useful in future nanoparticle design for biological applications.

The influence of contamination was studied with E_{int} and $E_{HOMO/LUMO}$ calculations on $C_{84}H_{24}$-NB, $C_{60}B_{12}N_{12}H_{24}$-NB and $C_{78}H_{24}FeN_4$-NB, where changes were observed in the presence of the contaminant ($B_{12}N_{12}$ and FeN_4). Significant binding for functionalized fullerenes was observed in HSA, HT2a and DNA systems, with the van der Waals and nonpolar terms being the dominant contributions. The detailed description of each investigation performed under NanoPUZZLES, including those which are mentioned above, can be found in [8, 10, 11, 42, 43].

10.3.3 Examples of Nano-QSAR Modelling

The NanoPUZZLES NanoQSAR work package developed nano-QSAR models based on the data and information discussed above, with the aim of extending the understanding of toxicity and behavior of nanoparticles by establishing relationships between experimental and computational properties.

To encode the cytotoxicity profile of metal oxide nanoparticles to the bacterium *E. coli*, periodic table-based descriptors were employed to construct robust interpretable quantitative structure-toxicity relationship (QSTR) models. Ten random splits of the data into the training and test set were examined with extensive validation techniques employing the Organization for Economic Co-operation and Development (OECD) recommendations. The results obtained have demonstrated that simple periodic table derived descriptors (metal electronegativity and the charge of the metal cation corresponding to a given oxide) have a significant influence on the cytotoxicity of metal oxide nanoparticles to bacteria *E. coli*. Both descriptors were obtained from the molecular formula and information derived from the periodic table. Moreover, both descriptors are independent of the nanoparticles' size range, thus avoiding the modelling difficulties arising with the variation of various physical nanoparticle properties with the size range. In addition, the results obtained confirmed that the toxicity of metal oxide nanoparticles is associated with the reductive potential, i.e., the detachment of the electron from the metal oxides [7].

To investigate the differences in the mechanisms of toxicity of metal oxide nanoparticles to the bacterium *E. coli* (prokaryotic system) and a human keratinocyte cell line (eukaryotic system), different computational approaches were utilized. To reflect the nanoparticles' structure for the different levels of organization, i.e. from single molecule to supramolecular ensemble of molecules, a Simplex Representation of Molecular Structure (SiRMS) and the "liquid drop" model (LDM) were used. Classification models were developed based on the combination of descriptors calculated within the NanoPUZZLES NanoDESC work package. Based on these simple fragmentary 2D descriptors (forming two latent variables) a consensus model was developed. The Nano-QSAR models obtained provided reliable pre-

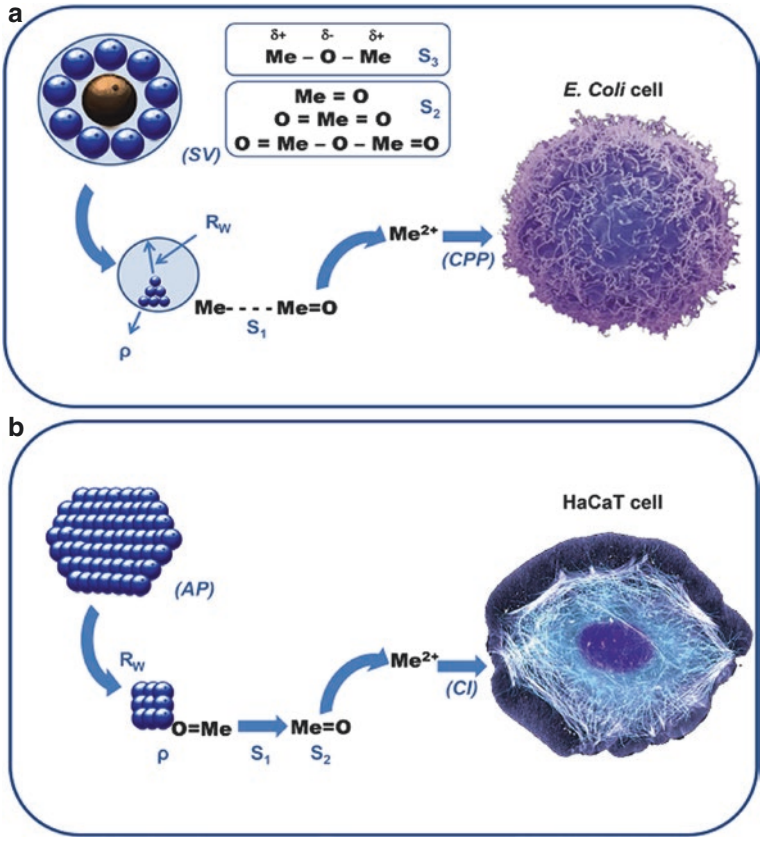

Fig. 10.5 Schematic representation of the mechanism of metal oxide nanoparticle toxicity for: (**a**) bacterium *E. coli* and (**b**) human keratinocyte cell line (HaCaT) (Reproduced from Sizochenko et al. [32], http://dx.doi.org/10.1039/c4nr03487b with permission of The Royal Society of Chemistry)

dictions for all metal oxide nanoparticles studied. It needs to be highlighted, however, that the developed nano-QSAR models refer to different mechanisms of toxicity of nanoparticles towards the bacterium *E. coli* and the human keratinocyte cell line (HaCaT) [30]. The schematic representation of suggested mechanisms is shown in Fig. 10.5.

As further examples, toxicological data collected and novel nanodescriptors calculated were used to model the cellular uptake of 109 magnetofluorescent nanoparticles modified with small organic molecules into $PaCa_2$ pancreatic cancer cells [38] and the membrane damage (units/L) caused by TiO_2 nanoparticles. The latter model is based on the use of eclectic information, in particular the following physicochemical parameters of the TiO_2 nanoparticles have been adopted: engineered size, size in water suspension, size in phosphate buffered saline, concentration, and zeta potential [39].

With nanomaterials there is the problem of the lack of standardization and harmonization, in addition to the related issue of finding suitable descriptors. The novelty of the CORAL approach, as applied to nano-QSAR, is in its simplicity [40]. It is highly flexible and involves a broad series of parameters of different nature, including experimental values arising from the different protocols. Thus, the model can adapt the features of interest depending on the case, covering a wide set of situations. CORAL is shown to be a general system and that several specific models can be produced through CORAL using the particular parameters relevant in each case. The disadvantage of this, which however is related to the lack of general protocols in many of the experiments with nanomaterials, is that the specific model requires as inputs the values of the specific experimental case used to train the model, and thus the specific model is very local.

Optimal descriptors calculated within NanoPUZZLES have also been used to develop a mathematical model for the mutagenicity of fullerenes. Experimental data for the bacterial reverse mutation test on C_{60} nanoparticles for TA100, and WP2uvrA/pkM101 were employed as dependent variables, i.e. endpoints. The models obtained were the mathematical function of all available eclectic data, such as: dose, metabolic activation (i.e. with or without S9 mix) and illumination (i.e. darkness or irradiation), in the role of logical and digital basis [36].

Moreover, optimal descriptors have been applied to predict thermal conductivity of micro-electro-mechanical systems (MEMS), largely used in nanotechnology. The decimal logarithm of thermal conductivity of MEMS has been modelled as a mathematical function of temperature and physicochemical status of MEMS, such as Ceramic (code = 1), Single crystal (code = 2), Cubic (code = 3), Chemical Vapour Deposition (CVD, code = 4), and Glass (code = 5) [41].

In summary, within NanoPUZZLES, physico-chemical properties as well as toxicity of a series of nanoparticles were predicted using various approaches. It was demonstrated that all models provide reliable predictions, though are characterized by different accuracy as compared to experimental data. The models developed allow for the prediction of physico-chemical properties and toxicity of new nanoparticles, not studied yet experimentally.

10.4 Nanoparticle Evaluation to Be Set in a Global Context

The evaluation of nanomaterials, toxicity tests and development of predictive models cannot be singular efforts but should be coordinated within ongoing global initiatives in order to make valid conclusions on nanoparticle toxicity. The presented work was integrated in international research and discussions, as shown in Fig. 10.6.

The NanoPUZZLES project coordinated approaches with other EU research projects modelling nanotoxicity (ModNanoTox, NanoTransKinetics, ModEnpTox, MembraneNanoPart, PreNanoTox, MODERN). At the European level, all projects addressing different aspects of nanosafety are joined under the overarching umbrella of the NanoSafety Cluster (http://www.nanosafetycluster.eu), with cross-cluster working groups on cross-cutting topics such as databases, hazard, risk and modeling.

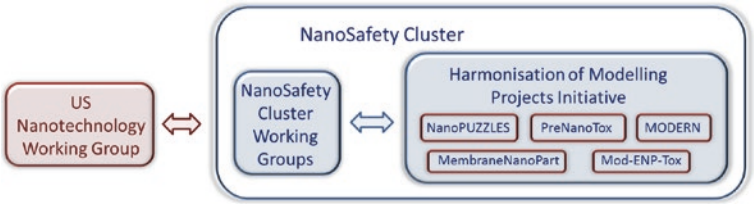

Fig. 10.6 Collaboration of the NanoPUZZLES project with other nanotoxicity modelling projects and setting within global nano-research networks

Furthermore, an EU-US dialogue (http://us-eu.org) framework is coordinating ongoing efforts and discussions on both sides of the Atlantic. In addition, the US Nanotechnology Working Group (https://wiki.nci.nih.gov/display/ICR/Nanotechnology+Working+Group), including the developers of the ISA-TAB-Nano specification, is very active for example in discussing data sharing standards, ontologies and nanomaterial characterisation.

10.5 Conclusions

Computational models predicting nanotoxicity could make an important contribution to safety evaluations, in line with general efforts in applying alternative methods in chemicals risk assessment, as promoted by recent legislation such as REACH and the European Cosmetics Regulation. This includes in silico models such as nano-QSARs for properties and toxicities.

Compiled, well organized, quality controlled and standardized information allows for the optimal use of existing data to support nanomaterial hazard and risk assessment. Integrating the data in an overarching, structured data collection pursues the following aims: a) organizing the data in a way to support the modelling, b) making certain that all (meta)data necessary for modelling are available, c) adding value to the available data by making a comparison between data from different sources possible. Databases of nanoparticle physico-chemical and toxicological properties thus allow for the use and comparison of all available data in integrated risk assessment approaches, avoiding new in vivo tests.

Based on the available data, specific descriptors can be derived to describe the nanomaterial-specific structure and physico-chemical properties appropriately. Furthermore, the interactions between nanoparticles and biological systems as well as small molecules, which can lead to modifications of the structure of the active nanoparticles, need to be described and taken into account in the development of models to predict the biological activity and toxicity of nanoparticles.

Overall, a global effort is required and is being built up to overcome the fragmentation of efforts to create, characterize and assess the toxicity data needed for the nanomaterial hazard and risk assessment. It aims to bridge the gap between experimental, computational as well as regulatory efforts.

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