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Toward a systematic exploration of nano-bio interactions

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Abstract

Many studies of nanomaterials make non-systematic alterations of nanoparticle physicochemical properties. Given the immense size of the property space for nanomaterials, such approaches are not very useful in elucidating fundamental relationships between inherent physicochemical properties of these materials and their interactions with, and effects on, biological systems. Data driven artificial intelligence methods such as machine learning algorithms have proven highly effective in generating models with good predictivity and some degree of interpretability. They can provide a viable method of reducing or eliminating animal testing. However, careful experimental design with the modelling of the results in mind is a proven and efficient way of exploring large materials spaces. This approach, coupled with high speed automated experimental synthesis and characterization technologies now appearing, is the fastest route to developing models that regulatory bodies may find useful. We advocate greatly increased focus on systematic modification of physicochemical properties of nanoparticles combined with comprehensive biological evaluation and computational analysis. This is essential to obtain better mechanistic understanding of nano-bio interactions, and to derive quantitatively predictive and robust models for the properties of nanomaterials that have useful domains of applicability.

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Transparency document

The Transparency document associated with this article can be found, in the online version.

1. Introduction

The commercial exploitation of the nanomaterials is continuing strongly, with many thousands of products now incorporating nano-scale forms of materials (Consumer Products Inventory, 2013). The commercial value of nanotechnology is estimated to be \$75Bn by 2020 (Mulvaney and Weiss, 2016). Nanotechnology is now used in diverse industrial applications, (Gurmu and Hazarika, 2011; Coelho et al., 2012; Jampilek and Krá ová, 2015) in biomedicine, (Dykman and Khlebtsov, 2012; Li et al., 2012) and in consumer products, (Wissing and Müller, 2003; Lem et al., 2012) amongst others.

Diagnostic (Frey et al., 2009; Hahn et al., 2011) and therapeutic (Brigger et al., 2002; Chatterjee et al., 2008) applications of nanomaterials involve direct injection or oral administration of nanoparticles. Release of nanoparticles from industrial (Kägi et al., 2008; Kaegi et al., 2010) and human activities (such as fires and automobile use) (Li et al., 2011), and heavy use of nanoparticle-based consumer products (Consumer Products Inventory, 2013) cause also lead to significant human exposure and environmental accumulation of nanoparticles (Gottschalk and Nowack, 2011; Keller and Lazareva, 2013). Nanoparticles or ultrafine particles that enter the human body via respiration, ingestion, or skin absorption (e.g. from cosmetics and sun-screens) can perturb normal functioning of physiological systems (Delfino et al., 2005; Geiser et al., 2005; Zhang et al., 2014). Consequently, how nanoparticles interact with biological systems has become an important, but very complex research and regulatory question. When nanoparticles encounter biomolecules or cells, their physicochemical properties have a major impact on their propensity to cause adverse biological perturbations (Albanese et al., 2012; Beckspeier et al., 2012; Braakhuis et al., 2014). However, how such effects are related to nanoparticle's physicochemical properties is far from being comprehensively understood.

The percentage of surface atoms, and the surface to volume ratio, increases when particle size decreases to the nanoscale. This generates more atomic or molecular interactions with biomolecules compared to larger particles. At the same time, the surface curvature affects how nanoparticles interact with biomolecules or cells. Some studies have shown that gold nanoparticles (Chithrani et al., 2006) and FITC coated mesoporous silica nanoparticles (Lu et al., 2009) with diameters of 40–50 nm are internalized by cells most readily. In other studies, smaller gold nanoparticles had the highest cell uptake (Chithrani et al., 2006; Walkey et al., 2012) and the most severe cytotoxicity (Vecchio et al., 2012). Uptake of nanoparticles also depends on the cell type interacting with the nanomaterial and the uptake mechanism used by the cells. Studies have shown that macrophages and other macrophage-like cells recognize and prefer to take up larger nanoparticles with sizes more similar to large proteins (> 100 nm for superparamagnetic iron oxide nanoparticles (SPIONs) and cellular debris that they normally process (Beduneau et al., 2009). For receptor-mediated endocytosis used by some cell types there is a 'sweet spot' in the size range of 30–50 nm, where the particle can engage a large number of receptors but is not too large to limit the binding of other nanoparticles (Albanese et al., 2012).

Shape is another property that modulates biological effects of nanoparticles. Nanoparticles with different shapes also have altered surface to volume ratios and different exposed crystal

faces. Such differences can regulate nanoparticle's interactions with biomolecules (Kodiha et al., 2014). Cellular uptake of nanospheres (14 and 74 nm) was 3–5 fold higher than that of nanorods (74 × 14 nm) (Chithrani et al., 2006). Uptake of rod shaped nanoparticles decreased with an increase of their aspect ratio (Chithrani et al., 2006; Chithrani and Chan, 2007). However, another study found that the cell uptake of nanorods (15 × 50 nm) was more efficient than nanospheres (15 and 50 nm) (Bartneck et al., 2010). A recent study showed that nanorods generated lower lactate dehydrogenase (LDH) release from human umbilical vein endothelial cells and human hepatocellular liver carcinoma cells but did not significantly affect other adverse biochemical endpoints (Le et al., 2016b). Consequently, the question of how nanoparticle shape affects biology, particularly adverse biological consequence such as cytotoxicity, is not clearly resolved and is likely to be cell type dependent (Schaeublin et al., 2012; Wan et al., 2015).

Nanoparticles made from different core materials can have a significant influence on their stabilities in an aqueous environment. For example, Ag nanoparticles release toxic Ag⁺ ions in aqueous solutions (Liu et al., 2010). Because of their different atomic radii and electronic arrangements, metal nanoparticles such as Au, Ag, Cu, Pt and Pd nanoparticles form different types of self-assembled monolayers on their surface when coated with thiolates (Love et al., 2005; Häkkinen, 2012). However, the binding modes, such as tilt angle and chain/plane rotation of the bound ligands, are very different (Love et al., 2005). These differences may have biological implications. Negatively charged silver, gold, and SPIONs also affect EGF signaling response in different ways (Comfort et al., 2011). Modelling of modified SPIONs also suggested that the type of iron core influenced the ability of these nanoparticles to cause apoptosis in smooth muscle cells (Epa et al., 2012). All these findings have elucidated the importance of the core material, especially where it can release of ions by dissolution, or when the core materials are redoxactive. (Puzyn et al., 2011; Yin and Casey, 2014).

However, binding of bovine serum albumin molecules by GNPs and AgNPs showed that protein adsorption is more related to surface modifications, not the core material (Treuel et al., 2010). Similarly, the uptake of SPIONs by macrophages depended more on the molecular weight of the dextran coating than the size of the nanoparticle, with lower molecular weights favouring uptake (Beduneau et al., 2009). Therefore, whether and how the core material or surface ligands dictate nanoparticle's biological activity is still unknown. Consequently, altering the surface chemistry provides opportunities to create nanoparticles with different hydrophobicity, charge density, π -electron density, or molecular geometry in order to start addressing this question. It is intuitively obvious that, because of their large surface to volume ratios, modifications to the surfaces of nanoparticles will significantly influence nano-bio interactions. Many studies have reported that charged nanoparticles adsorb more proteins than neutral nanoparticles e.g. (Deng et al., 2012), and hydrophobic nanoparticles are more easily internalized by cells than hydrophilic nanoparticles e.g. (Fytianos et al., 2015). Furthermore, positively charged GNPs have higher cellular uptake and much higher cytotoxicity than negatively charged nanoparticles (Fröhlich, 2012) because of stronger interactions between positively charged GNPs and the negatively charged lipid bilayers. A similar phenomenon is seen with cationic molecular species such

as antiseptics like chlorhexidine, where bilayer destabilization occurs in bacteria (Ikeda et al., 1984; Wernert et al., 2004), and with antimicrobial peptides. (Andersson et al., 2016).

Clearly, the number of properties that can be altered in nanoparticles is relatively large, generating a potentially huge combinatorial space of possibilities. For example, if a nanoparticle is synthesized with 20 different core materials, 10 different particle diameters, 5 possible dopants with concentration ranges varying in 10 steps, and 5 different types of surface coatings, this generates 50,000 different materials. If the surface chemistry is varied by addition of 500 types of small organic molecules, the size of the materials space is now 25 million nanomaterials. Additional environmental processes like homo- and hetero-aggregation, ionization of surface functionality at different pH values, the adsorption of proteins, ions, and organic molecules to the surface of the particles etc. add enormously to the complexity of the 'biologically relevant entity'.

Unfortunately, reported investigations on physical, compositional, and surface effects are mainly non-systematic and largely limited in scope. These studies also generally compare the effect of varying a single property at a time, while neglecting changes in all others or holding the others constant. However, biological effects are usually results of combined changes in multiple properties of nanoparticles. Few published studies involve systematic variation of several nanoparticle properties simultaneously (see Le et al. (2016b) for a recent example). Nano-bio interactions may also be affected by the specific nanoparticle preparation methods, cell types, the biological environment, experimental conditions, assay method, and ageing (dissolution and agglomeration). These additional complications make comparisons of results generated by different labs more difficult. To fill the gaps in our understanding of nano-bio interactions, more systematic research approaches need to be developed. The reasons why this is important, and how it may be achieved, is the main focus of this perspective.

2. Systematic chemical and physicochemical modifications

As mentioned, the high specific surface area of nanoparticles underscores the greater impact of surface modifications on their biological effects. Systematic chemical modifications can be achieved by multiple and simultaneous surface modifications, or by gradual alteration of only one property while keeping other properties essentially unchanged (e.g. univariate gradient studies). The importance of systematic exploration of physicochemical parameter spaces is identified in the small but increasing number of studies in which more parameters are varied simultaneously (Fig. 1). Nel et al. described how such nanomaterials libraries and high throughput toxicological screens may be designed (Nel, 2012). Lohse et al. recently reported a millifluidic benchtop device that could synthesize libraries of functionalized nanomaterials in a high throughput manner (Lohse et al., 2013). In a similar vein, Liu et al. described a versatile and robust microfluidic platform for synthesizing diverse types of homogeneous nanoparticles with different physical properties and drug loadings (Liu et al., 2015a). The method could generate up to 240 g/day of nanoparticles.

Akin to the exploration of chemical space using small molecule combinatorial libraries (Zhou et al., 2008b), nanoparticle surface chemistry can be modified using a combinatorial

chemistry approach. To assemble diverse nano-combinatorial libraries, molecular building blocks can be selected by computational and experimental design methods to have different physicochemical parameters (Lynn et al., 2001; Akinc et al., 2003). Chemically diverse nano-combinatorial libraries in which multiple properties are varied are also most amenable to computational modelling approaches such as quantitative structure-activity relationship (QSAR) analysis (sometimes called nano-QSAR when used to model nanomaterials properties). For example, Zhou et al. showed that surface molecular diversity in a combinatorial multiwall carbon nanotube library modulated cytotoxicity (Zhou et al., 2008a), immunotoxicity (Gao et al., 2011), autophagy (Fig. 2a) (Wu et al., 2014), cell differentiation (Zhang et al., 2012), and perturbations to the CYP3A4 enzyme in the liver (Fig. 2b) (Zhang et al., 2016) in systematic ways. QSAR analysis of the cytotoxicity data generated a computational prediction model that facilitated nanotube design (Fourches et al., 2015). A nano-combinatorial gold nanoparticle library aids discovery of cell-specific and high affinity binding nanoparticles that can distinguish between related cell types, so have potential applications in diagnostics and personalized medicine (Zhou et al., 2010; Le et al., 2015).

A different systematic approach is to use incremental or continuous changes in only one property while other properties are kept constant (e.g. univariate gradient studies). When gold nanoparticles with gradually increasing positive or negative charges were incubated with cells, they were internalized by cells only when their surface positive charge density reached a certain threshold (Fig. 3a) (Su et al., 2012). In contrast, when the gold nanoparticles were synthesized to have gradual increase in hydrophobicity, cell uptake and cytotoxicity increased with increasing experimental LogP values (an experimental surrogate for the lipophilicity or polarity of a material) (Fig. 3b) (Li et al., 2015).

Previous univariate approaches ignore the enormous size of nanomaterials composition and physicochemical spaces, and the complex interplay between properties that drive biological interactions (Le and Winkler, 2015). The number of drug-like molecules that could be synthesized has been estimated at 10^{60} , while estimates of the number of accessible materials range to 10^{80} , numbers so large they are for all practical purposes, infinite. Consequently, for nanomaterials there is an urgent need to study and model multiple physicochemical properties, and their possible interactions (i.e. multivariate studies) to best capture the complexity and nonlinearity in the relationships between nanomaterial microscopic and physicochemical properties and their impact on biological systems.

Adopting developments in robotics and automation to explore nanomaterials properties faster has been recognized for some time (Winkler et al., 2013). Not only can large parallel experiments generate data more quickly and allow the effects of nanoparticle properties on biology to be elucidated, when used in conjunction with appropriately sparse design of experiments (DoS) methods, they allow the generation of computational models that can recapitulate the entire response surface. Taken together these methods also show considerable promise in leveraging existing animal data more broadly, and for reducing or eliminating animal testing in the future. It has even been possible to use in vitro data in concert with molecular descriptors to improve predictive, computational models of in vivo responses. (Lee et al., 2010) These technologies also work synergistically with evolutionary

methods (Le and Winkler, 2016) that aim to explore large regions of compositional or other parameter spaces, to optimize a particular set of biological properties. The technology now exists to assemble relatively large combinatorial nanoparticle libraries in which chemical and physical properties are changed simultaneously (e.g. Fig. 4). This approach has a high probability of success, and work on such complex libraries is in progress in our laboratories, and in several others. Nano-bio interaction data sets generated in these studies will be much larger than those in previous published research. Clearly, such nano-bio “big data” sets can only be properly evaluated using computational approaches (Winkler et al., 2013; Winkler, 2015). The size of these data sets, particularly those that may involve the effects of nanomaterials on gene expression, also creates a strong need for robust data management and informatics tools to guarantee data integrity, record provenance, provide relevant annotation, and to optimize accessibility.

The above discussion has focused on the nanoparticle properties over which the experimenter has direct control. There are clearly a large number of other transformations that nanoparticles undergo that are largely environment-specific and that generate the ‘biologically relevant entity’ that interacts directly with cells, interfaces, and other biological systems. Many of these, such as the dynamic behaviour of protein coronas as a function of biological compartment, are very important but not yet well understood. Systematic laboratory studies of the interaction of nanoparticles with specific common serum and plasma proteins would help elucidate these interactions, and may allow the corona compositions in different types of biological environments to be modelled and predicted as a function of the concentration of soluble proteins and as a function of time. These studies are starting to appear in the literature, with relatively high throughput methods becoming available for identifying proteins in coronas of different types of nanoparticles in a range of biological media. For example, Duran et al. (Duran et al., 2015) and Eigenheer and coworkers (Eigenheer et al., 2014) have studied how particle size and surface modification affect the nature of the protein coronas of silver nanoparticles. Lilia Barran-Berdon et al. reported the experimental determination of the time evolution of nanoparticle corona on exposure to human plasma, (Lilia Barran-Berdon et al., 2013) and Darabi Sahneh et al. have begun to develop mathematical models that predict the dynamics of protein attachment to nanoparticles (Darabi Sahneh et al., 2013). Proteomics and lipidomics are increasingly being used to understand the composition of coronas in different environments. (Pozzi et al., 2015; Raesch et al., 2015) Such differences in the corona composition critically affect subsequent nanoparticle pathophysiology (Tenzer et al., 2013). Systematic studies of the effect of inorganic ions, the effect of pH on surface charge of chemically functionalized nanoparticles, are probably less challenging to conduct, but would yield important information that would ultimately allow the interaction of specific nanoparticles with a given environment to be predicted computationally. Promising early work is starting to appear in the literature. Recently Garbovskiy reported studies of the adsorption/ desorption of ions from nanoparticles, and Kallay and Zalac described their models for the kinetics of aggregation of nanoparticles. (Kallay and Zalac, 2002; Garbovskiy, 2016) Properties related to nanoparticle ageing, specifically agglomeration and aqueous solubility, are other clear candidates for a systematic approach to experiments and modelling. If systematic studies of these nanoparticle properties yield enough information on the mechanisms at play, it will

allow these properties to be deliberately controlled, providing rational design of bespoke 'biologically relevant entities' for specific applications in diagnostics, therapeutics, delivery, or safe-by-design materials.

3. Nano-bio interaction data, nanoinformatics, and modelling

Research in nanotechnology is critically dependent on good management, sharing, and computational interpretation of nano-bio interaction data (Panneerselvam and Choi, 2014). An increasing number of publicly available databases exist, such as the NBI knowledgebase (NBI Knowledgebase, 2015), caNanoLab (caNanoLab, 2015), Nanomaterial Registry (Nanomaterial Registry, 2015), ISA-TAB-Nano (ISA-TAB-Nano, 2015), OECD Safety of Manufactured Nanomaterials (OECD Safety of Manufactured Nanomaterials, 2015) and Nano-EHS Database Analysis Tool (Nano-EHS Database Analysis Tool, 2015). These databases focus on different protocols (data sources, experimental protocols and literature), diverse users (medical researchers, scientists, and the general public), and different data sharing goals (harmonizing data, accessing data, and data formats). Therefore, data formats are quite varied and data quality is not entirely consistent. Work has started on the development of data ontologies (a system for formal naming and definition of the types, properties, and interrelationships of entities) such as eNanoMapper (Hastings et al., 2015), and a consistent markup language (a system for annotating a document in a way that is syntactically distinguishable from the text) (Erkimbaev et al., 2015) to describe nanomaterials data. Once operational, it will allow data exchange between groups, applications, and instruments in a robust and transparent way. Currently, due to lack of these universally accepted protocols, some confidentiality issues, the relative fragmentation of international nanotechnology research (COST Actions and other international networking efforts notwithstanding), and the complexity of nanomaterials relative to discrete molecules, the availability of public data for nanoparticles is still quite limited compared to the data sources for small molecules, see for example (Wang et al., 2010). Recent efforts to use high throughput screening techniques (Gangwal et al., 2011) and nano-combinatorial nanomaterial library design (Lynn et al., 2001; Akinc et al., 2003; Zhou et al., 2008a; Zhou et al., 2010; Pankova et al., 2015) lead the way for the future enrichment of public data sources for nanoparticles.

As with the development of data collection and management methodologies, computational modelling of nanoparticles is still at a relatively early stage. The complexity of nanomaterials and their interactions with biological environments, cells and other entities has limited physics-based modelling efforts (e.g. using quantum chemical or molecular dynamics methods) to specific idealized model systems, such as simple physicochemical properties and environments (e.g. pristine nanoparticles in water). Machine learning and other data-driven statistical modelling methods have shown great promise for modelling complex nanomaterials (Winkler et al., 2013; Winkler, 2015; Le et al., 2016a), but the abovementioned paucity of reliable data sets is the *main limitation* in making rapid progress. Most modelling studies of nano-bio interactions have been based on QSAR methods, with substantially fewer molecular dynamics studies being reported, always modelling systems that are in far from realistic nanomaterials environments. The most relevant variables in these modelling studies were particle size (Powers et al., 2007; Park et al., 2011), shape

(Poland et al., 2008), crystal structure information (Napierska et al., 2010), surface charge (Park et al., 2013), and surface chemistry (Glötzer and Solomon, 2007; Xia et al., 2010).

QSAR and molecular dynamics models can provide useful information on important nanostructure properties that determine biological effects. Data driven machine learning or other statistical models can make robust predictions of biological properties of nanoparticles not yet synthesized or tested, a capability that regulatory agencies need (Gajewicz et al., 2012). Most studies so far use the QSAR modelling method to link the nanostructures descriptors to their bioactivities, and to make quantitative predictions of the biological properties of materials not used to train the models. Publications reporting reliable, predictive, data-driven modelling of nanoparticle properties and toxicities are still relatively scarce, and they are often derived from small datasets with relatively few physicochemical properties being probed (Meng et al., 2009). These models consequently have quite limited domains of applicability, making reliable prediction of the properties of nanoparticles in different regions of compositional or property space impossible or at least unreliable (see also Le et al., Gramatica, and Alexander et al. for methods to quantify the robustness, predictivity, and quality of statistical QSAR models (Gramatica, 2007; Le et al., 2012; Alexander et al., 2015)). We emphasize that the *methodology* to generate high quality computational models that can predict the properties of new materials is well established, it is the lack of *experimental design* in the generation of the experimental data, and the *paucity of data* itself that is the main roadblock (Winkler et al., 2013) to rapid progress in computational prediction of the biological properties of nanomaterials. Proper use of experimental design can sample moderate regions of experimental space with relatively few experiments.

Puzyn et al. published one of the earliest nano-QSAR modelling studies that used just a single chemical descriptor, the formation enthalpy of a gaseous cation with the same oxidation state, to generate a very good model of cytotoxicity of nanoparticles (Puzyn et al., 2011). This parameter is highly correlated with the ionization potential of the oxidation state of the metal and thus to redox properties (Le et al., 2016b). Fourches et al. studied various nanoparticles (Fourches et al., 2010; Fourches et al., 2011) and reported the first experimentally validated computer prediction (Fourches et al., 2015). A recent study by Liu et al. developed a QSAR model based on the atomization energy of the metal oxide, period of the nanoparticle metal, and nanoparticle primary size (Liu et al., 2011). Recent advances and still unresolved issues in the application of computational modelling of nano-bio interactions have been reviewed recently (Winkler, 2015). Computational models of corona composition and its effect on biological activity, using molecular dynamics methods, nonlinear dynamics equations, or machine learning have also been reported recently (Darabi Sahneh et al., 2013; Papa et al., 2016).

Despite the great promise of data driven methods, the limitation in most studies is the *small size of data sets* of nanoparticles (e.g. usually <20). The limited availability of much larger biological data sets that could be used to train models has meant that a significant number of published studies use the same data sets generated by a few labs (Weissleder et al., 2005; Shaw et al., 2008; Zhou et al., 2008a; Zhang et al., 2009; Zhou et al., 2010). Compared to small molecule chemical descriptors developed for QSAR models of drug candidates, the

size and complexity of nanostructures and the fact that nanoparticles exist as distributions rather than discrete entities make the development of general nano-specific descriptors *very difficult*. Moreover, due to the importance and complexity of the surface microenvironment, new types of nano-descriptors are urgently needed to be developed. For example, Li et al. recently simulated the accessibility of water molecules near functionalized nanoparticle surface ligands by modelling nano-hydrophobicity (Li et al., 2015). In the study, the traditional chemical descriptors were weighed by the accessibility of water molecules to fit the condition of nanostructures. Development of nano-specific descriptors is clearly an important but relatively neglected area of research.

Modelling studies are also severely limited by the *quality* of nanomaterial preparation. For example, it will be difficult to calculate nano-descriptors precisely without experimentally measuring the size, shape, ligand density and distribution for surface modified nanomaterials. Likewise, nanomaterials are often not well characterized in terms of size distributions, shapes, degree of agglomeration and coverage of coatings, introducing further errors and uncertainties into the modelling process. Recent published work has highlighted the potential for machine learning models to facilitate purposeful design of nanomaterials with bespoke properties, particularly for medical applications or to minimize adverse biological effects. (Fourches et al., 2015; Le et al., 2015; Li et al., 2015; Liu et al., 2015b; Zhang et al., 2016). Fourches et al., for example, used a computational model to virtually screen external libraries and design new carbon nanotubes with favoured properties. Subsequent experimental validation showed the usefulness of the resulting model in predicting the properties of new nanomaterials (Fourches et al., 2015). With these initial successes and proof of concept, it is expected that nano informatics models will make an increasingly strong contribution to the design and regulation of nanomaterials within this decade (Winkler et al., 2013).

4. Concluding remarks

Understanding nano-bio interactions is much more complex than comprehending the molecular interactions of small molecules with protein targets (although these are complicated enough). There is a paucity of systematic studies and modelling of nano-bio interactions that has slowed progress in the field. Although the need for accelerated synthesis and testing methods for nanomaterials was identified almost a decade ago, progress toward this important goal has been slow. Experimental methods like protein NMR, and small molecule/protein co-crystallization cannot yet be reliably used for nanoparticles, or indeed be used at all.

Fortunately, paradigm shifts in robotics and automation, coupled with the development of more effective machine learning algorithms like deep learning (Winkler and Le, 2016) have provided a synergistic environment for greatly expanding systematic studies of nano-bio interactions. Increasingly, high throughput nanoparticle library synthesis, biophysical characterization and biological testing methods are being used to generate large data sets relating to nano-bio interactions at the biomolecule or cellular levels. However, analysis of these data and generation of computational models leveraging it to make predictions about new materials with superior properties is not trivial. In order to take maximum advantage of

these potentially disruptive technologies in systematic nanoparticle structure-property studies, significant research needs to be done into more effective ways of mathematically describing these nanoparticle and target properties. Taken together, the combination of rapid synthesis and testing, robust and predictive modelling based on new modelling paradigms and better descriptors will allow a systematic exploration of nano-bio interactions across many dimensions. This will provide a framework for rational design of safer and more effective nanomaterials, a mechanistic (possibly molecular level) understanding of the interactions between nanomaterials and biological systems, and a suite of effective computational tools that are urgently needed by regulators to control the safe use of these fascinating and highly useful materials.

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Fig. 1.

Nanomaterials compositional and combinatorial libraries that could be used to generate mechanism-based toxicological data suitable for use in models linking nanomaterials properties to biological impact.

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Fig. 2.

Heat-maps of autophagy and CYP3A4 perturbation induced by a combinatorial multiwalled carbon nanotubes library. (a) Heat-map of autophagy induction in U87 cell by modified MWCNTs ranked from basal to high levels. (b) CYP3A4 perturbation is modulated by modified MWCNTs in human liver microsomes as determined by degradation of a drug nifedipine. Adapted with permission from reference (Wu et al., 2014), copyright 2014 American Chemical Society and (Zhang et al., 2016), copyright 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Fig. 3.

The influence of surface charge density and hydrophobicity of nanoparticles on cell uptake. (a) Cell uptake of GNPs (25 mg mL⁻¹) with a continuous change in surface charge density in HeLa cells after a 12 h incubation. (b) Cell uptake of GNPs (25 mg mL⁻¹) with a change in experimental LogP in (PMA)-induced THP-1 macrophages after a 24 h incubation. Adapted with permission from reference (Su et al., 2012), copyright 2012 American Chemical Society, and reference (Li et al., 2015), copyright 2015 Elsevier Ltd.

Fig. 4. Nano-combinatorial chemistry approach with consideration of both physical and chemical modulations.