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Modelling and predicting the biological effects of nanomaterials

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The commercial applications of nanoparticles are growing rapidly, but we know relatively little about how nanoparticles interact with biological systems. Their value – but also their risk – is related to their nanophase properties being markedly different to those of the same material in bulk. Experiments to determine how nanoparticles are taken up, distributed, modified, and elicit any adverse effects are essential. However, cost and time considerations mean that predictive models would also be extremely valuable, particularly assisting regulators to minimize health and environmental risks.

We used novel sparse machine learning methods that employ Bayesian neural networks to model three nanoparticle data sets using both linear and nonlinear machine learning methods. The first data comprised iron oxide nanoparticles decorated with 108 different molecules tested against five cell lines, HUVEC, pancreatic cancer, and three macrophage or macrophage-like lines. The second data set comprised 52 nanoparticles with various core compositions, coatings, and surface attachments. The nanoparticles were characterized using four descriptors (size, relaxivities, and zeta potential), and their biological effects on four cells lines assessed using four biological assays per cell line and four concentrations per assay. The third data set involved the biological responses to gold nanoparticles functionalized by 80 different small molecules. Nonspecific binding and binding to AChE were the biological endpoints modelled. The biological effects of nanoparticles were modelled using molecular descriptors for the molecules that decorated the nanoparticle surface. Models with good statistical quality were constructed for most biological endpoints. These proof-of-concept models show that modelling biological effects of nanomaterials is possible using modern modelling methods.

Keywords: nanomaterials; adverse biological effects; QSAR; modelling and prediction; neural network; Bayesian methods

1. Introduction

Nanomaterials have generated a vast amount of research interest in the past decade due to the unique properties that nano forms of materials possess, largely because of their very high surface areas. These novel properties of nanomaterials have seen their rapid incorporation into products (an estimated 2000 product types containing nanomaterials by 2015, with an estimated market value of 1 trillion dollars, see http://www.nanotechproject.org/cpi/about/analysis/ and http://www.epa.gov/nanoscience/quickfinder/nanomaterials.htm).

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1.1 *Adverse effects of nanoparticles*

Life on earth has been exposed to nanoparticles in the environment since it evolved. Natural sources of nanoparticles include fires, dust, volcanoes, colloidal processes, etc. Mechanisms for dealing with adverse biological impacts of nanoparticles have been part of the natural evolution of living organisms so, in many cases, nanoparticles of natural origin can be tolerated with little or no impact. Consequently, it is possible that many manufactured or engineered nanoparticles, or those generated adventitiously during manufacturing processes, may similarly be dealt with by living organisms with little adverse impact.

However, it is known that some types of nanoparticles are definitely toxic [1]. There are myriad serious diseases that are caused by exposure to nanomaterials (Figure 1) [2]. While the mechanisms responsible for the serious effects of asbestos or silica in nanoparticulate form are well known, for many other types of nanoparticles this is not the case. Many adverse biological effects in humans and the environment are not known because the materials have been developed only recently, and the relevant experiments have not yet been done.

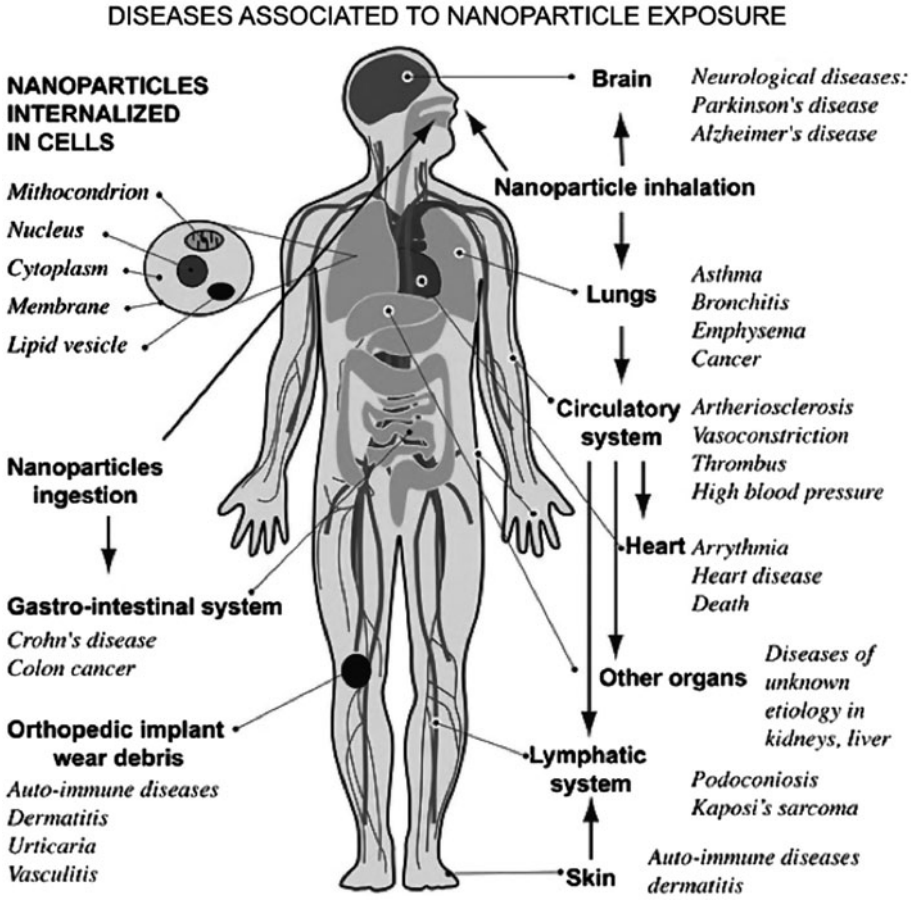


Figure 1. Diseases associated with nanoparticle exposure (Buzea et al. [2], Wiki Commons).

1.2 *Why computational modelling?*

Computational modelling of the properties of nanomaterials allows prediction of biological effects. New feature selection and machine learning methods are robust and widely applicable. Computational models are very fast and can make predictions on materials not yet used or even synthesized. They promise to provide tools to help governments regulate nanomaterials safely without stifling their commercial development.

Computational models have the following advantages [3]:

- Experimental testing of chemicals for physicochemical, toxicological, and environmental properties is time-consuming and expensive and cannot keep up with the very large number of nano products being developed.
- There is increasing pressure to reduce or discontinue animal testing.
- Computational methods like QSAR have become increasingly useful and reliable, and are now accepted by regulators for the assessment of industrial chemicals (REACH, NICNAS etc.) [4,5].
- Computational modelling will complement, not replace, the need for experimental assessment of the biological effects of nanoparticles. However, it is data driven and requires high-throughput nanoparticle synthesis and assessment methods.

A recent workshop on the application of QSAR approaches to modelling and prediction defined a roadmap for progress in the field that will provide computational models and tools that regulators can use to make decisions about the regulation of nanomaterials. This workshop, sponsored by the European Science and Technology agency COST, identified the key experiments and computational developments that would be necessary to derive useful models of the biological effects of nanomaterials in a timely manner [6].

1.3 *Problems we face – can we do anything?*

The issues involved with modelling the biological effects of nanoparticles are substantial. The most immediate and important problems revolve around the heterogeneous distribution of nanoparticles' sizes and shapes, difficulties in characterizing their properties, lack of suitable descriptors to define their properties, complex interactions with biological fluids in animals or humans, or with environmental substances, that result in the formation of a complex corona around the particle, and general lack of knowledge of how nanoparticles are taken up by cells, are distributed around the body, metabolized or biologically processed and excreted. That is, nanoparticles are heterogeneous and poorly defined compared with pure organic compounds, they tend to aggregate or agglomerate, and they interact strongly with, and are transformed by, their environment. The wealth of biochemical and pharmacological knowledge on how small organic molecules interact with living systems is lacking for nanoparticles. Nonetheless, regulators need decision support tools for nanomaterials [6].

1.4 *Modelling the biological effects of nanomaterials*

Given the difficulties in dealing with nanoparticles, and the general paucity of mechanistic knowledge on how they interact with biological systems, machine learning, QSAR-based modelling approaches are the only viable approaches at this stage [7].

They are data-driven, supervised modelling methods that capture the complex relationships between the molecular (microscopic) and physicochemical properties of nanomaterials

and their biological (macroscopic) effects without requiring detailed knowledge of the mechanisms of interaction.

$$\text{Biological response (BR)} = F(\text{molecular properties}) \quad (1)$$

Although this approach is the most promising one, difficulties arise from lack of nano-specific descriptors and the paucity of large data sets to train models. Hopefully the increasing use of combinatorial and high-throughput materials experimentation will solve the second problem soon. The development of nano-specific descriptors is an important research issue for the field. Recent development in the mathematics of feature selection, and nonlinear regression, together with suitable descriptors, allows the derivation of the most appropriate form for the (usually nonlinear) function $F = g(\text{corona}) \times h(\text{uptake}) \times j(\text{mechanism}) \times k(\text{bio-processing})(...)$. These robust pattern recognition methods can accommodate complex 'models within models' and generate useful predictive models for these complex materials.

The 50 years since the QSAR method was developed have provided a wealth of experience and tools that can be brought to bear on the prediction of the properties of nanomaterials, and materials more generally [7]. We illustrate the potential of QSAR machine learning approaches to model the biological effects of nanoparticles with three examples.

2. Materials and methods

The materials and data for the three examples were taken from the original source publications [8–10].

2.1 *Nanomaterials*

The nanoparticles for the smooth muscle apoptosis and cellular uptake experiments were iron oxide particles with an average diameter of 30 nm and aspect ratios close to 1. They were coated with natural (dextran) or synthetic polymers that are chemically cross-linked (CLIO, cross-linked iron oxide), then covalently functionalized with small organic molecules. The compositions of the nanoparticles, their surface coatings, surface functionalization, and measured properties are summarized in the publications of Weissleder et al. [9] and Epa et al. [11]. The third data set consisted of gold nanoparticles whose surfaces were functionalized by small organic molecules [10]. TEM analysis showed that diameters of the gold nanoparticles were around 5–6 nm and they were dispersed well in water with very little aggregation. The chemical composition of the surfaces of the gold nanoparticles is summarized in Figure 2.

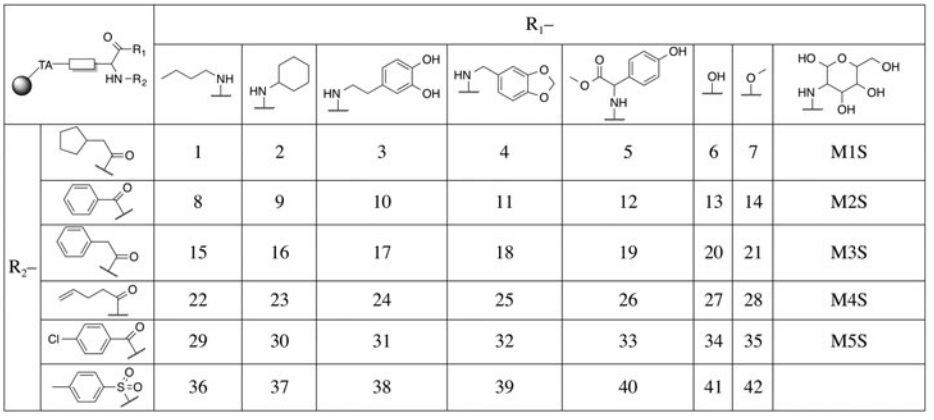
The modified surface chemistry was originally aimed at targeting the nanoparticle to specific cell types for use in diagnostics or therapeutic medical applications. These materials were chosen because the surface chemistry was the dominant feature of the nanoparticles, and the small molecule coatings were more easily characterized by existing descriptors.

2.2 *Biological measurements*

Biological data were taken from the original source papers or were modified according to recent publications.

2.2.1 *Cellular apoptosis induced by CLIO nanoparticles*

Shaw et al. [8] reported a study on the effects of 50 different nanoparticles in four cell lines (endothelial and smooth muscle cells, monocytes, and hepatocytes), using four biological



		R1–							
R2–		1	2	3	4	5	6	7	M1S
		8	9	10	11	12	13	14	M2S
		15	16	17	18	19	20	21	M3S
		22	23	24	25	26	27	28	M4S
		29	30	31	32	33	34	35	M5S
		36	37	38	39	40	41	42	

Figure 2. Surface functionalization of gold nanoparticles. The surface chemistry is defined by the combinatorial reaction of R_1 with R_2.

assays (ATP content, reducing equivalents, caspase-mediated apoptosis, and mitochondrial membrane potential) in each cell line, at four concentrations per assay. These experiments generated potentially 64 biological response variables for each of the 50 nanoparticles (3200 data points). As the data did not allow an EC_{50} or similar parameter to be calculated from the dose-response data, we used the slope of the dose-response curve (effectively the Hill slope) as a dependent variable in the analyses. We generated models either using all of the data in the model, or splitting the data into a training set of 26, and a test set of six nanoparticles using a *k*-means clustering method.

2.2.2 *Cellular uptake of surface-modified superparamagnetic iron oxide nanoparticles*

Weissleder et al. [9] screened a library of 109 fluorescent nanoparticles sharing a common superparamagnetic iron oxide (SPIO) core and dextran coating, whose surfaces were conjugated with diverse small molecules. Nanoparticles were evaluated for cellular uptake (in human umbilical vein endothelial cells (HUVEC), primary resting human macrophages (RestMph), granulocyte macrophage colony stimulating factor-stimulated human macrophages (GMCSF_Mph), a U937 human macrophage-like cell line (U937), and human pancreatic ductal adenocarcinoma cells (PaCa2)), measured by well fluorescein isothiocyanate (FITC) concentrations. Experimental data were used as its $\log_{10}$ transform, and clustering was used to divide it into a test set of 21 molecules (i.e. 20% of the data) and a training set of 87 molecules (80% of the data).

In assessing whether both of the above assays contain useful biological information, the *z*-scored data were used where $z^{NP} = (\mu_{NP}-\mu_{PBS})/\sigma_{PBS}$, where μ and σ are the mean and standard deviation of assay replicates, respectively, and the NP and PBS subscripts represent assays in the presence of PBS buffer controls. Assays where most of the dose-response curves fell within a *z*-score of ±2 were considered to demonstrate negligible effect.

2.2.3 *Protein adsorption on surface-modified gold nanoparticles*

The protein adsorption was determined in terms of the quenching of fluorescence of aromatic surface residues such as tryptophans, tyrosines and phenylalanines, which is directly

correlated with the amount of protein [10]. Specific binding to acetylcholinesterase was determined by the degree of inhibition of the enzymatic activity.

2.3 *Machine learning methods and descriptors*

Given the complexity of the materials and the environments in which they were placed, we used general, robust, sparse linear and nonlinear modelling methods. We also employed sparse Bayesian linear and nonlinear feature selection methods to optimize the complexity and predictivity of models. The sparse linear feature selection and regression was performed using a multiple linear regression plus expectation minimization algorithm with a sparse (Laplacian) prior [12]. Sparse nonlinear feature selection was performed using a Bayesian regularized neural network with either a Gaussian or Laplacian prior [13–15]. The latter method simultaneously carries out nonlinear feature selection and weight pruning to optimize the sparsity and predictivity of the resulting models. The neural net models used a three-layer feed-forward neural network. The input layer contained the same number of nodes as descriptors, the hidden layer generally two or three nodes, and the output layer a single node corresponding to the property being modelled. The input and output nodes contained linear transfer functions, and the hidden layer a sigmoidal function. The networks were trained using a Levenberg–Marquardt algorithm until a maximum in the Bayesian evidence was achieved. Unlike standard backpropagation neural networks, Bayesian regularized networks do not need a validation set to determine when training should stop. They also generate models that are relatively insensitive to the number of hidden layer modes, making optimization of their architecture simple [13]. The cellular uptake and protein adsorption models employed 2D DRAGON descriptors [16], excluding 3D and quantum chemistry-derived descriptors. While a QSAR model should ideally be predictive and also interpretable in terms of molecular interactions between the material and the biological system, given the chemical diversity of nanoparticle surfaces, the unknown composition of protein corona and lack of mechanistic information on uptake etc., it is not possible at this stage to make such detailed mechanistic interpretations.

3. Results and discussion

3.1 *Cellular apoptosis induced by CLIO nanoparticles*

Only the apoptosis assays [8] exhibited significant dose-response relationships, with the nanoparticles generating changes in the other biological assays that were not statistically significant. The smooth muscle cell apoptosis assay generated the most statistically significant models. The type of nanoparticle core material (Fe_2O_3 versus Fe_3O_4), surface coating type, and the surface charge influenced smooth muscle apoptosis. Consequently, we developed models using three indicator variables for these three properties: nature of the nanoparticle core (+1 for Fe_2O_3 and 0 for Fe_3O_4); type of coating (+1 for dextran and 0 for other coatings); and nature of surface functionality (encoded as +1 (basic), −1 (acidic), or 0 (neutral)) [11].

This yielded a simple but statistically significant QSAR equation that predicted smooth muscle apoptosis (SMA) induced by the metal oxide nanoparticles:

$$\mathrm{SMA} + 2.26(\pm 0.72) - 10.73(\pm 1.05)I_{\mathrm{Fe2O3}} - 5.57(\pm 0.98)I_{\mathrm{dextran}} - 3.53(\pm 0.54)I_{\mathrm{charge}} \quad (2)$$

The model statistics were as follows: training set squared correlation coefficient $r^2_{\mathrm{train}} = 0.81$; test set regression coefficient $r^2_{\mathrm{test}} = 0.86$; standard error of estimation (SEE) = 3.6; and of external prediction (SEP) = 3.3. We also derived statistically significant nonlinear models

with the following model statistics: r^2_{train} = 0.80; test set regression coefficient r^2_{test} = 0.90; standard error of estimation (SEE) = 2.8; and of external prediction (SEP) = 2.9. The model used six effective weights and generated smaller prediction errors for both training and test data than the linear model [11].

Some biological interpretation of these simple models is possible. The weak dependence of the cell apoptosis on the surface charge indicator variable or zeta potential of the particles is probably due to adsorption of a protein corona in plasma or serum (present in cell culture media). This protein coat or corona generates an effective zeta potential of a relatively constant –10 to –20 mV, so it is not unexpected that the surface charge-related descriptors of pristine nanoparticles are not as relevant to the models. The strongest factor affecting SMA was the nanoparticle core material, which is probably related to the differences in available Fe(II) and Fe(III) in the iron oxide nanoparticles (Fe_2O_3 has only Fe(III) and Fe_3O_4 has Fe(II) and Fe(III) present) and their ability to generate reactive oxygen species (ROS) and cellular cytotoxic and proinflammatory responses [11].

3.2 *Selective uptake of surface-modified SPIO nanoparticles*

The cellular uptake data reported by Weissleder et al. [9] was for 109 superparamagnetic and dextran-coated nanoparticles that were decorated by different small molecules conjugated to their surfaces. Of the five cell lines tested, only the PaCa2 and HUVEC lines showed significant variation in uptake as a function of surface modification. The three macrophage or macrophage-like cell lines showed little variation in uptake surface chemistry (Figure 3).

Linear and nonlinear QSAR models using DRAGON descriptors could successfully predict the uptake of nanoparticles in PaCa and HUVEC cell lines for the training and test sets (Table 1) [11]. The linear and nonlinear models had similar predictive power.

The nanoparticle uptake by HUVEC and PaCa2 cells could be predicted to within a factor of 2 (Table 1 and Figure 4).

There was almost no overlap in the identity of the 11 descriptors in the HUVEC model and the 19 descriptors in the PaCa2 model, suggesting that different uptake mechanisms may be in play [11]. The poor response of the macrophage and macrophage-like cells to nanoparticle uptake may be due to the small size of the nanoparticle (30 nm). As universal phagocytes,

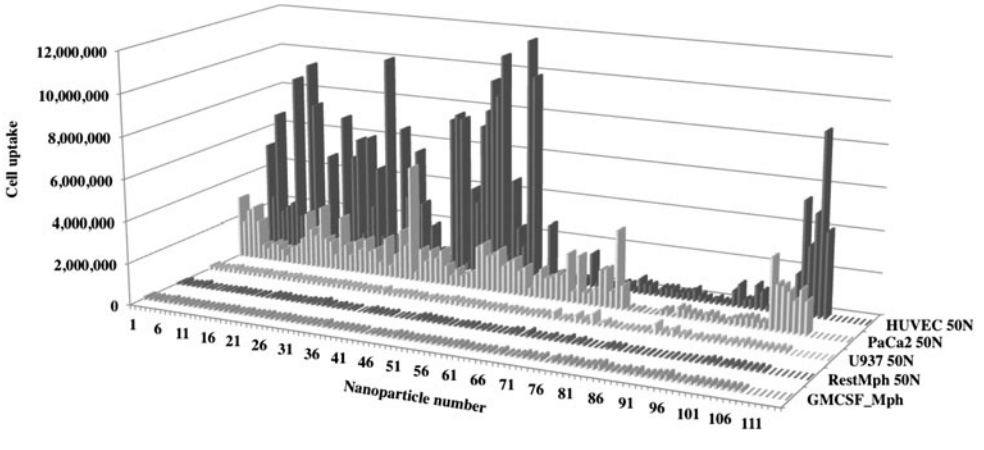


Figure 3. Degree of uptake of surface modified nanoparticles in different cell lines.

Table 1. Statistics for the QSAR models for the nanoparticle uptake.

Cell type	*Model*	*No. descriptors*	r^2_{train}	*SEE*	r^2_{test}	*SEP*
HUVEC	linear	11	0.74	0.34	0.63	0.36
	nonlinear	11	0.70	0.30	0.66	0.33
PaCa2	linear	19	0.76	0.19	0.79	0.24
	nonlinear	19	0.77	0.15	0.54	0.28

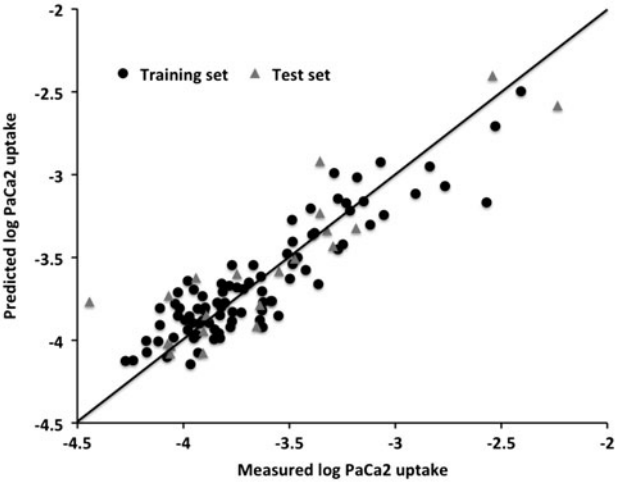


Figure 4. Predicted versus measured log nanoparticle uptake for PaCa cells. Training set (circles), test set (triangles).

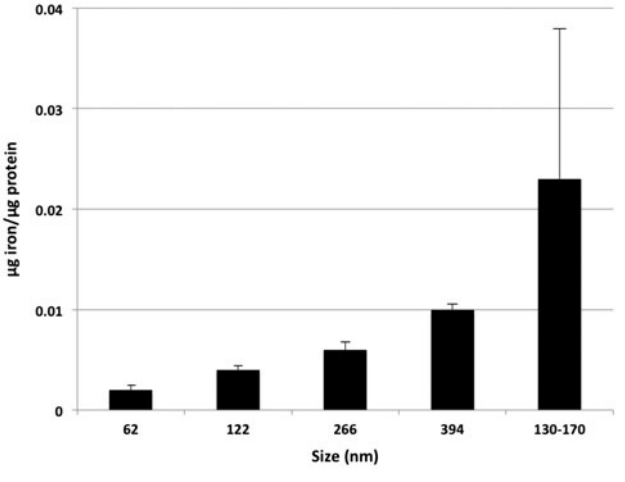


Figure 5. Uptake of ion nanoparticles by macrophages as a function of size (Beduneau et al. [17]).

macrophages generally recognize larger materials such as proteins. The uptake of iron oxide nanoparticles has been shown to increase dramatically above particle sizes of 300 nm [17] (Figure 5). Clearly, we also cannot rule out that modification of the nanoparticle surface chemistry may also influence uptake by macrophages.

3.3 *Protein binding to modified gold nanoparticles*

Yan's group measured the nonspecific adsorption and acetylcholinesterase (AChE) inhibition by 47 surface-modified gold nanoparticles [10]. The variation of adsorption with surface chemistry is illustrated in Figure 6.

We modelled the AChE inhibition or nonspecific adsorption using linear and nonlinear models and 14 DRAGON descriptors. The best multiple linear regression (MLR) model for AChE inhibition using a test set of 20% of the data had an $r^2_{\text{train}} = 0.91$, SEE = 5.1 $r^2_{\text{test}} = 0.81$, and SEP = 5.6. The best Bayesian neural net model employed two nodes in the hidden layer and had $r^2_{\text{train}} = 0.84$, SEE = 3.7, $r^2_{\text{test}} = 0.8$, SEP = 5.2 and 14 effective weights in the model (Figure 7).

For nonspecific protein binding the models used 10 DRAGON descriptors and 20% of the data in the test set. The best MLR model had an $r^2_{\text{train}} = 0.93$, SEE = 0.88, $r^2_{\text{test}} = 0.93$, and SEP = 0.75. The best nonlinear Bayesian neural network model used two nodes in the hidden layer and had an $r^2_{\text{train}} = 0.96$, SEE = 0.43, $r^2_{\text{test}} = 0.94$, SEP = 0.75, and 15 effective weights in the model (Figure 8).

Clearly, these machine learning methods could successfully model several biological effects of surface-functionalized nanoparticles. They could predict the relevant biological effects of nanoparticles in independent test sets with similar accuracy as for the training set. As these are necessarily data-driven methods, they cannot obviate the need for experimental measurement; models are synergistic with measurements. Although these methods are relatively easy to apply, they cannot generate good predictive models from very small data sets, poor quality data, or poor descriptors. Although the prediction of properties of compounds in the independent test set was good, care must be taken to ensure that new predictions are not made too far from the property space in which they were derived (the domain of applicability of the models). This domain is defined by the span of chemical diversity used to generate the models and, correspondingly, by the ranges of the molecular descriptors and biological response data in the training sets. Data quality, quantity, diversity, range, relevance are paramount.

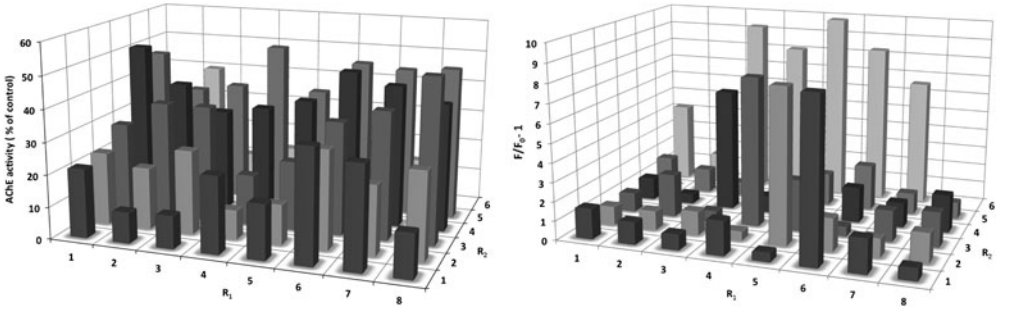


Figure 6. Left: The inhibition of AChE's activity by GNPs. Experimental conditions: final conc. of Au was 3 μg/mL and the AChE was 0.3 nM. Right: The fluorescence quenching of AChE by GNPs. Experimental conditions: AChE 1.67 μM in 0.1 M PBS (pH~8.0), final conc. of Au in AChE solution was 17 ppm.

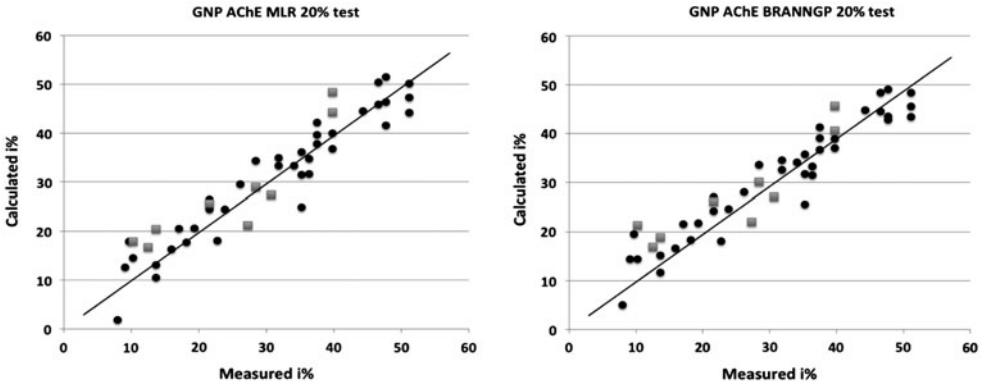


Figure 7. Measured and predicted AChE inhibition by surface modified gold nanoparticles for linear model (left), and nonlinear model (right). Training set (circles), test set (squares).

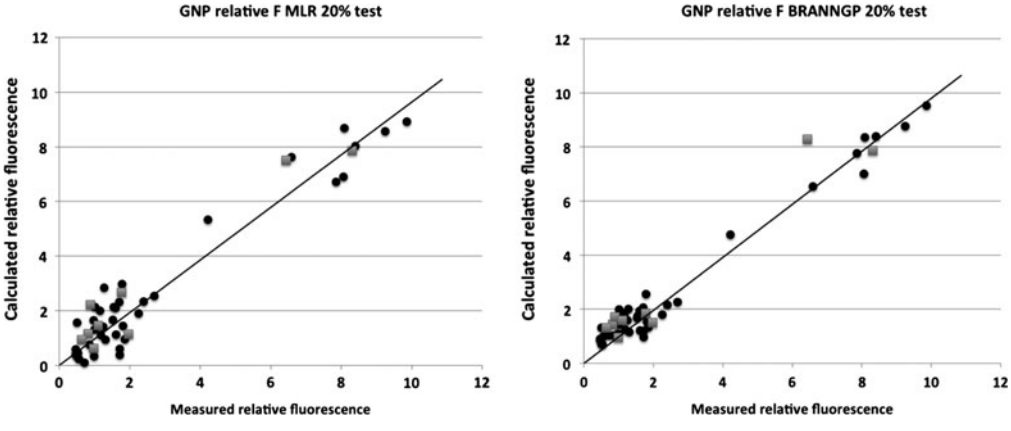


Figure 8. Measured and predicted nonspecific protein binding by surface-modified gold nanoparticles for linear model (left), and nonlinear model (right). Training set (circles), test set (squares).

To convince regulators and other science professionals that these models are useful it is important that their predictivity is tested experimentally. Molecular details of the mechanism of action are often not accessible from these models for the reasons summarized above. However, QSAR methods can capture complex relationships between structure and biological activity, even for multiple modes of action that are likely to prevail with nanoparticles interacting with biology.

4. Conclusions

We have illustrated the potential of machine learning methods to generate robust and predictive models of several biological effects of nanoparticles, and indeed materials more generally [7]. Although additional new nano-specific descriptors must be developed, the modelling techniques are intrinsically effective at modelling structure–activity relationships of very

complex materials. These methods are very fast and can deal with very large data sets, so are well matched to the increasing use of high-throughput nanomaterials, synthesis, characterization, and testing paradigms. They will play an important role in predicting the biological effects of nanomaterials in the future and, as with industrial chemicals, will provide useful models to assist regulators legislate to protect workers, the public and the environment without unnecessarily stifling innovation and commercial exploitation of nanomaterials.

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