Inexpensive and Accurate – Novel Computational Methods for Prediction of Toxicity of Nanomaterials

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Acknowledgements

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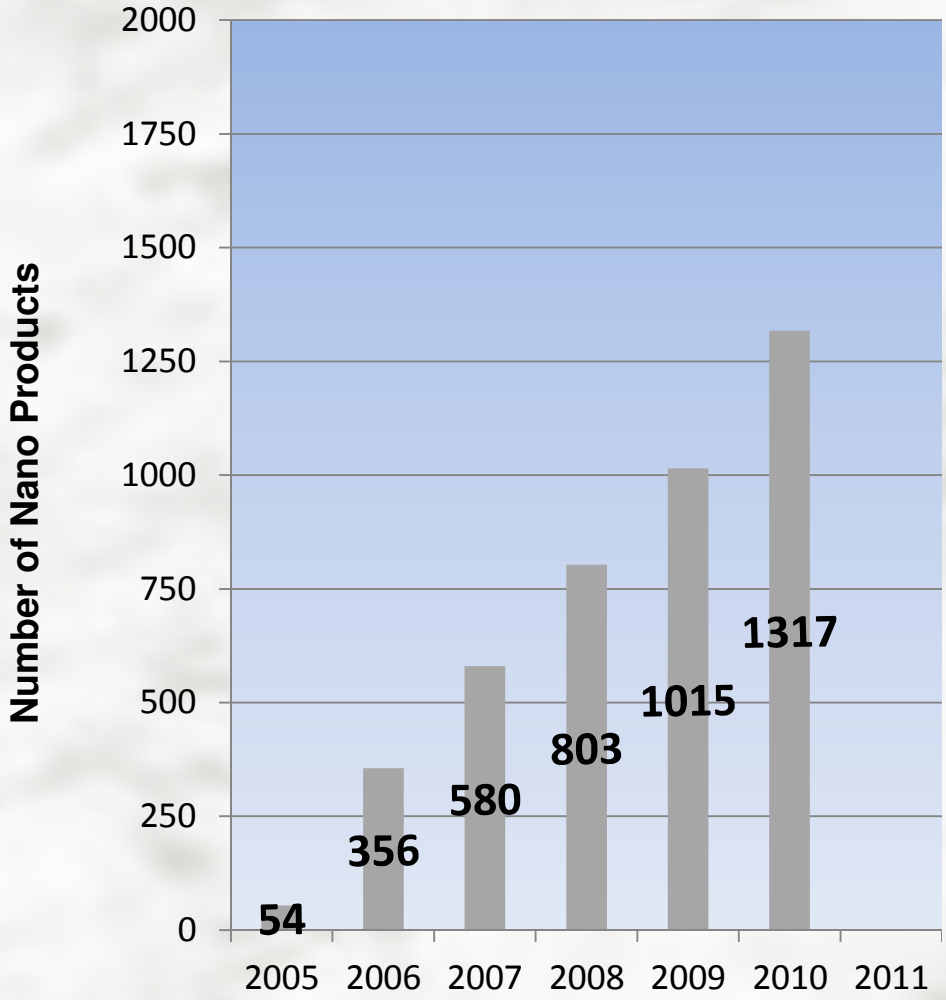
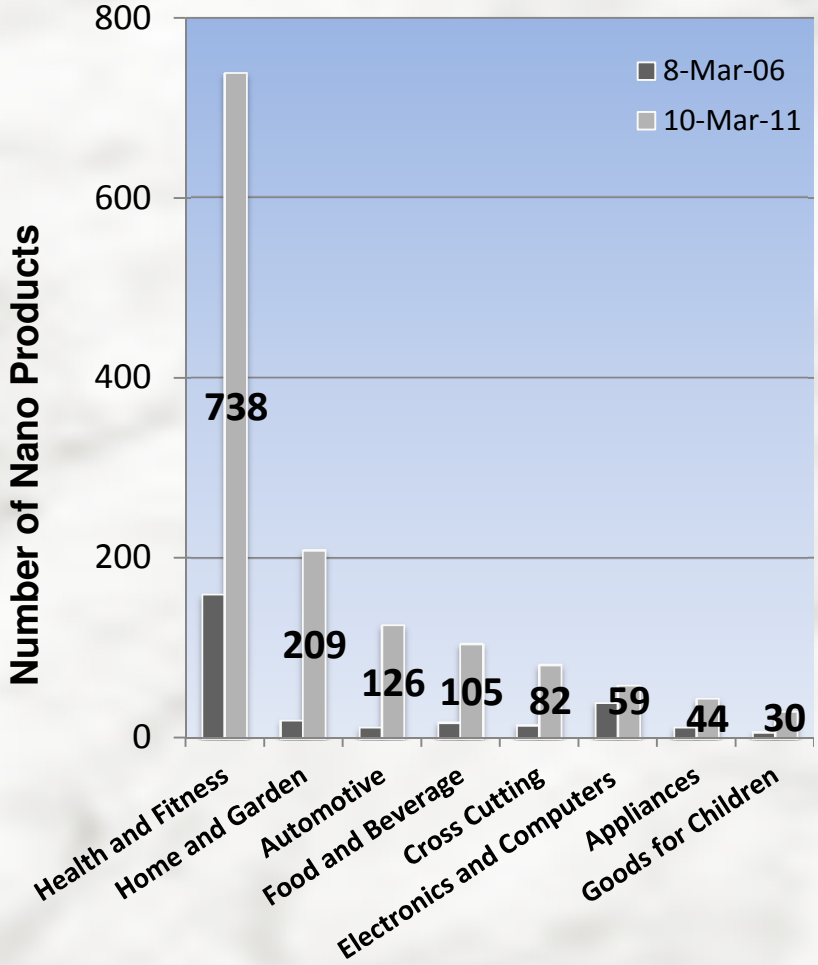
Total Nano Products Listed



Nano Product Categories



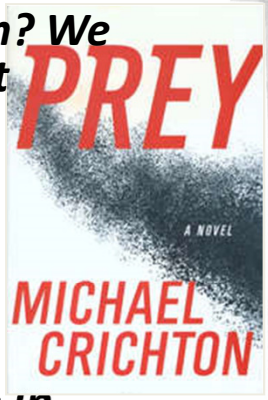
Source: http://www.nanotechproject.org/inventories/consumer/analysis_draft/

"What effect does it have on the environment? What happens if they don't break down? How do we get rid of them? We don't want to find out in 20 years that it causes cancer."

"[Nanotechnology] could create weapons worse than nuclear."

"I have reservations as far as its use in food, animals, in the chain that we eat."

Respondents from Survey – Woodrow Wilson International Center for Scholars



NATIONAL NEWS

THE WASHINGTON POST

Stricter Nanotechnology Laws Are Urged

Report Warns Of Risk to Public

By Rick Weiss
Washington Post Staff Writer

An independent report being released this morning concludes that current U.S. laws and regulations cannot adequately protect the public against the risks of nanotechnology — the rapidly growing science of making invisibly small particles and molecular devices.

Unless existing laws are modified or a new one is crafted, the report warns, the immense promise of the field — predicted to be a trillion-dollar industry by 2015 — may be short-circuited by either a disaster or an economically damaging crisis of public confidence.

"There is a chance to still do this right and learn from previous mistakes," said study author J. Clarence Davies, an environmental policy analyst who played major roles in the Johnson, Nixon and first Bush administrations and is now with Resources for the Future, a nonpartisan think tank on environmental and energy issues.

"We know from what happened with agricultural biotechnology and nuclear power that if you don't have public support, or at least public tolerance, a field's potential is not going to be realized. For nanotechnology, I don't think existing systems or laws can serve this purpose," said Davies, who researched and wrote the report for the Project on Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars, a research and policy arm of the Smithsonian Institution.

Several government officials and industry representatives disputed the findings yesterday. Among them was E. Clayton Teague, director of the National Nanotechnology Coordinating Office, which oversees the federal government's approximately $1 billion annual investment in nanotechnology.

"We still have so much to learn," he said. "You get one paper that says it's extremely toxic and harmful, and another that says it's not only not toxic but it's beneficial. All the agencies we talk with . . . have generally said to us that with the information that's currently available, their regulatory authorities should be adequate."

Nanotechnology involves manipulating atoms to make things that are smaller than one one-thousandth the diameter of a human hair. At that size, even conventional materials exhibit unconventional physical and chemical properties, making them valuable for a wide variety of products. Already, nanomaterials are being used in computers, cosmetics, stain-resistant fabrics, sports equipment, paints and medical diagnostic tests. Scientists also hope to use nanomaterials to help clean up polluted sites.

But nanoparticles' peculiar characteristics are potentially hazardous. Animal studies have shown that at least some can cause deadly airway blockages or can migrate from nasal passages into the brain and other organs, where they may cause metabolic problems. Other studies suggest they can trigger environmental damage that would be difficult to reverse once the minuscule particles disperse into soil and water.

The report outlines the range of laws and regulations in place to protect people and the environment from such risks, and finds each one wanting when it comes to nanotechnology.

The Toxic Substances Control Act (TSCA), for example, requires manufacturers to tell the Environmental Protection Agency about new chemicals they want to market and gives the agency the authority to restrict those that pose undue risks.

But under TSCA's "low volume" clause, chemicals made in quantities of 11 tons or less are largely exempt. That may be reasonable for conventional chemicals, the report states. But given the extreme chemical reactivity of nanomaterials — the very trait that makes them so special — the mere fact that relatively small quantities are being made is hardly an assurance of safety, it concludes.

Moreover, because most nanomaterials are ordinary chemicals that differ only in their particle size, makers have been avoiding TSCA restrictions by classifying their products as conventional chemicals, even though their tiny size is precisely what makes them different. The EPA, which favors a "voluntary" regulatory regimen for nanotechnology, has not yet decided how to deal with that loophole.

Other laws are weak because they do not require safety studies before products are marketed, Davies said. The Federal Hazardous Substances Act and the provisions of the Food, Drug and Cosmetic Act that deal with cosmetics — many of which are made with nano-ingredients — allow the government to respond only after it is clear that people are being harmed.

Moreover, the report notes, the agencies in charge of implementing the nation's protective laws are understaffed. The Occupational Safety and Health Administration has 25 percent fewer employees than it did in 1980, even though it must oversee more workplaces than ever — including nanotech factories with their uncertain risks — Davies said. The Consumer Product Safety Commission, which administers the Hazardous Substances Act, today employs 446 people, less than half the number there in 1980.

The Food and Drug Administration yesterday reiterated that existing regulations are "probably adequate for most nanotechnology products."

"We are continuing to learn about the important scientific developments in this area and will evaluate and learn about the benefits this new technology will yield," the agency said in a statement.

David Rejeski, who leads the Woodrow Wilson Center's nanoscience program, warned that nanoscale materials are increasingly being enhanced with biologically active coatings that will only increase their health and environmental risks. He said it would help if companies were required to tell regulators more about what they are up to.

"The behaviors of these substances are going to get very complicated," Rejeski said. "But there hasn't been a big discussion yet. We're data-starved."

Technology
Every Wednesday

Scared of new nano-pants? Hey, you may be onto something

In the late 1950s, my Uncle Jim and his teenage buddies would sometimes roam downtown Binghamton, N.Y., and stop at a little shoe store that had its very own X-ray machine.

It was the latest technology for getting your shoe size. Customers would come in, flick a switch, stick their feet in, and see how their foot bones lined up on a sizing chart. My uncle and his friends did this for kicks. The thing probably spit out hundreds or thousands of times the dosage you'd get from a dental X-ray today.

It's a wonder Uncle Jim never grew a few extra toes.

Contrast that with the naked folks in Chicago a few weeks ago.

By Kevin Maney

A handful of young men and women filed into an Eddie Bauer store and took off their clothes to protest the selling of khaki pants treated with nanotechnology.

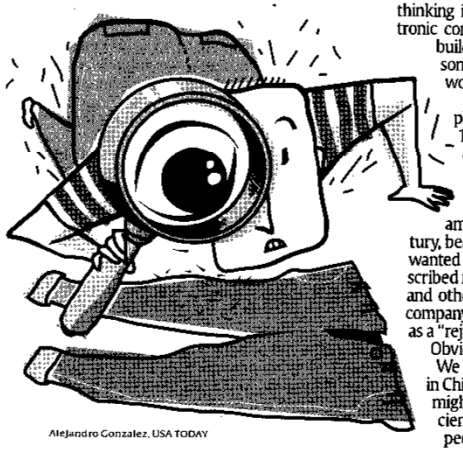
So far, there seems to be no reason to think anyone could be hurt by nano-pants, but a lot of people are terribly worried about nanotechnology. They've heard stories that it could self-replicate until it covers the Earth like a virulent kudzu, or that nanotech particles might damage brain cells or cause cancer. They're associated with the Institute of Electrical and Electronics Engineers Society on Social Implications of Technology. "The guy selling the innovation is often optimistic. But there's often this fear, and the fear is not entirely groundless."

In fact, IEEE preaches that technologists should welcome the protesters and skeptics because they force issues to the surface early, before something gets out of hand and causes widespread damage. The fears push technologies to improve, and get society to look at consequences and decide what trade-offs are acceptable.

"You always need both camps" — the optimists and the pessimists, says Brian O'Connell, current president of the Society on Social Implications of Technology.

Feelings about technology can also wax and wane with eras. In the 1920s, anything scientific and modern was seen as progress, and progress was good. In the 1960s, the space race lit up a generation of tech believers. In the 1990s, we all thought the Internet was going to "change everything" and swore it was the most significant human development since the Sumerians invented writing.

The crummy economies of the 1930s, 1970s and early 2000s wiped out a lot of that smiley-faced buoy-



AleJandro Gonzalez, USA TODAY

thinking it would threaten their jobs. The first electronic computers stirred fears that companies were building "electric brains." IBM CEO Thomas Watson Sr. made speeches saying the machines would never be able to think.

Then there's the flip side. In the 1940s, people labeled DDT the wonder pesticide. By the 1960s, Rachel Carlson published *Silent Spring*, alleging that DDT caused cancer and other environmental problems. The stuff was banned in the USA in 1973.

The story of X-rays and other radiation is among the most bizarre. In the early 20th century, beauty shops used doses of X-rays to make unwanted facial and body hair fall out. Physicians prescribed radioactive radium for heart trouble, arthritis and other ailments. For a while in Europe, a candy company marketed chocolate bars laced with radium as a "rejuvenator."

Obviously, none of that was a very good idea.

We really can't tell whether the naked protesters in Chicago are flakes or prophets. Nanotechnology might turn out to be like natural gas — an efficient, safe technology that benefits millions of people. Or it could be this generation's X-ray, and our grandchildren will guffaw at our na-

Chemical Inventory and Toxicological Testing in USA

•In USA, National Toxicology Program (NTP) is responsible to evaluate chemical agents having public health concern.

•Other agencies e.g. Environmental Protection Agency (EPA) and National Institute of Occupational Safety and Health (NIOSH) also have an important role.

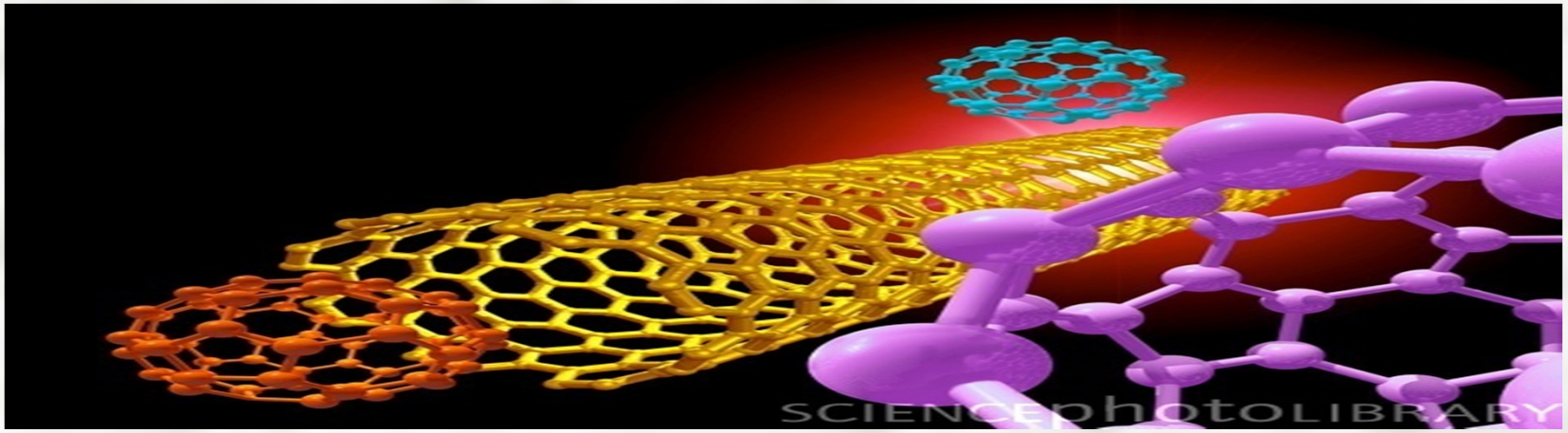
•There are about 82000 chemicals currently registered in USA for commercial use.

•Only 350 have undergone long-term and 70 short-term testing by NTP.

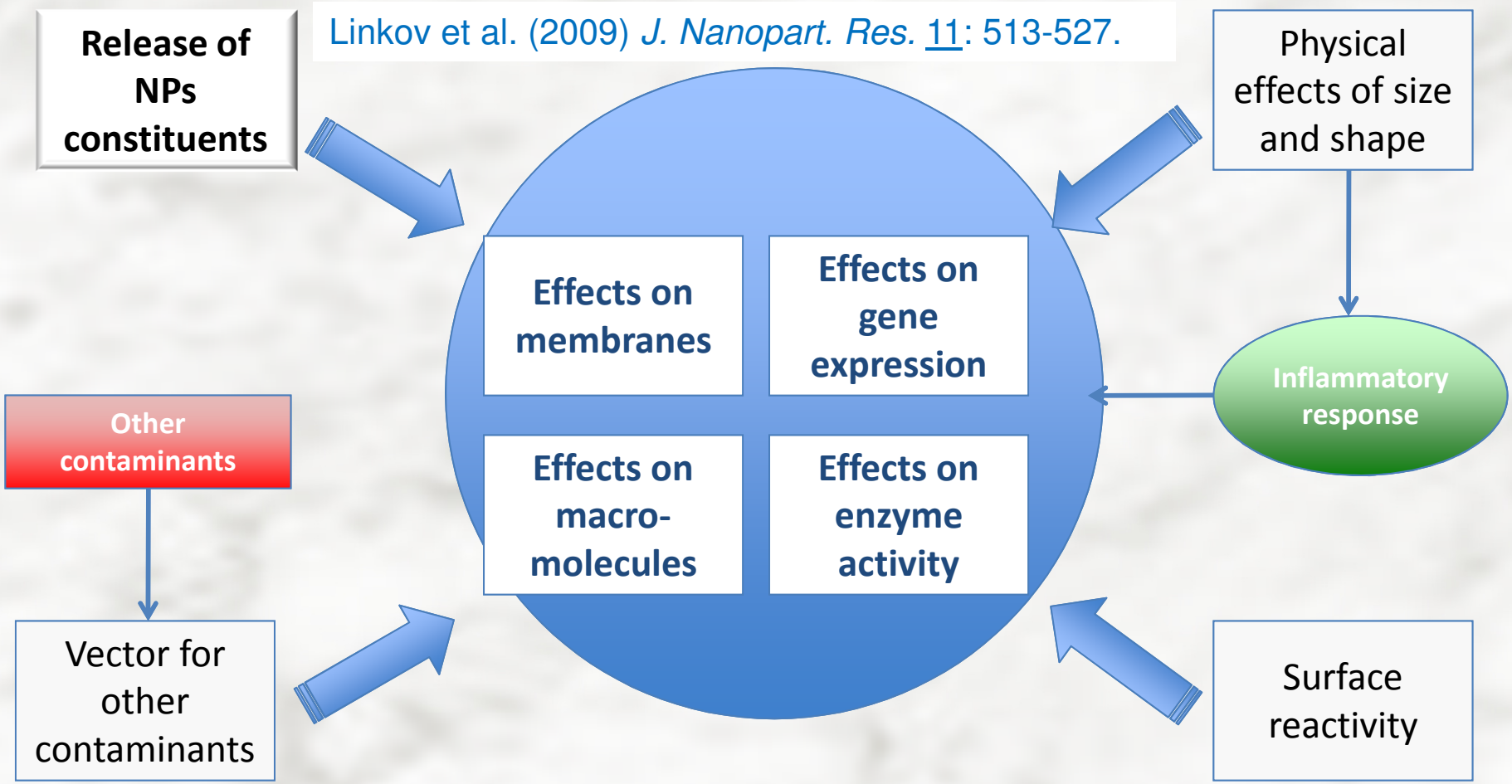
•Testing of each bioassay costs $2-4 million and over 3 years to complete test.

•Thus, in total about $160-320 billion and 240 thousand years total time will be needed to test chemicals currently in use.

Challenges of Nanomaterials: Are we on the way to comprehend their toxicity?

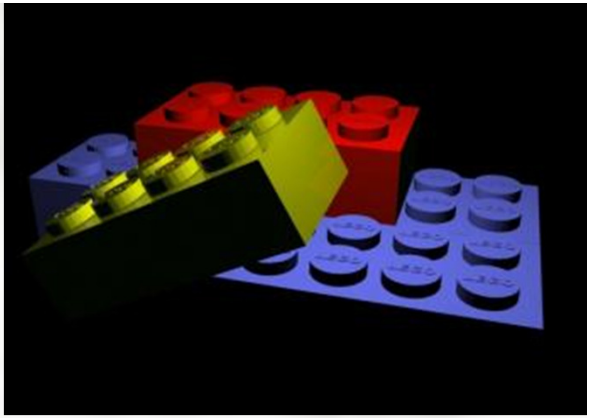


Postulated mechanisms of NPs' toxicity



Reality and Models

in silico

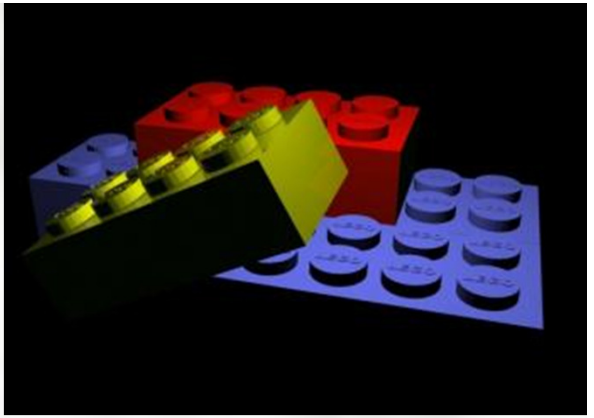


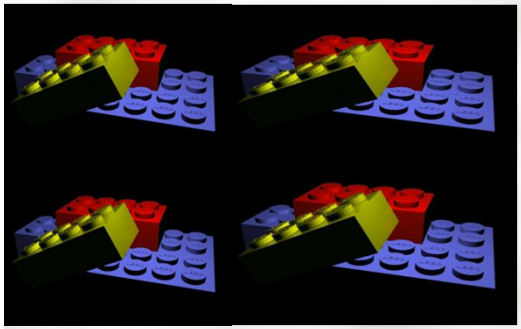
↕

in vivo

One More Step

in silico





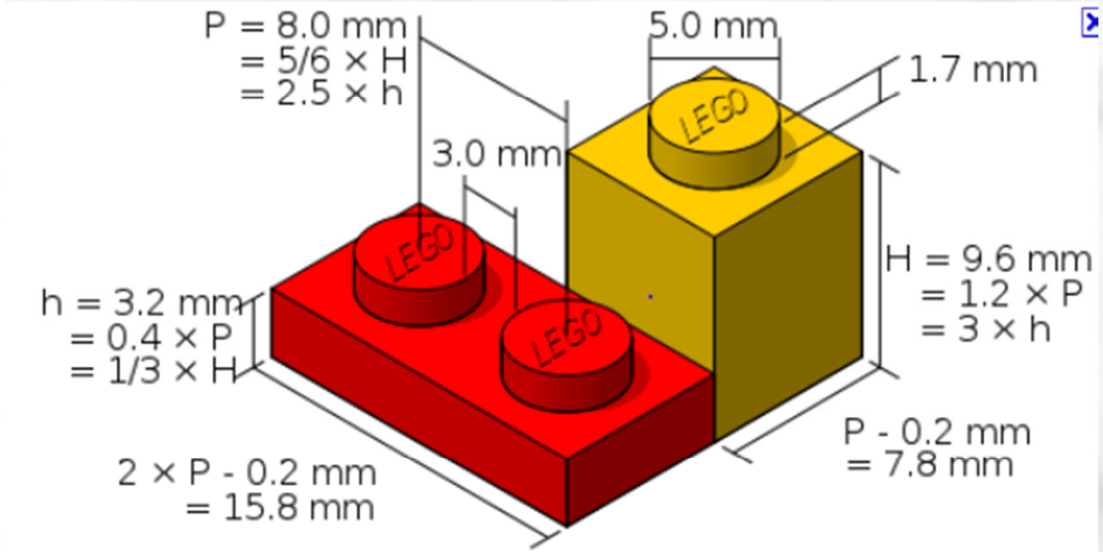
↕

in vitro

↕

in vivo

Does it Fit?


P = 8.0 mm
= 5/6 × H
= 2.5 × h
5.0 mm
1.7 mm
3.0 mm
H = 9.6 mm
= 1.2 × P
= 3 × h
h = 3.2 mm
= 0.4 × P
= 1/3 × H
P - 0.2 mm
= 7.8 mm
2 × P - 0.2 mm
= 15.8 mm
LEGO
LEGO
LEGO

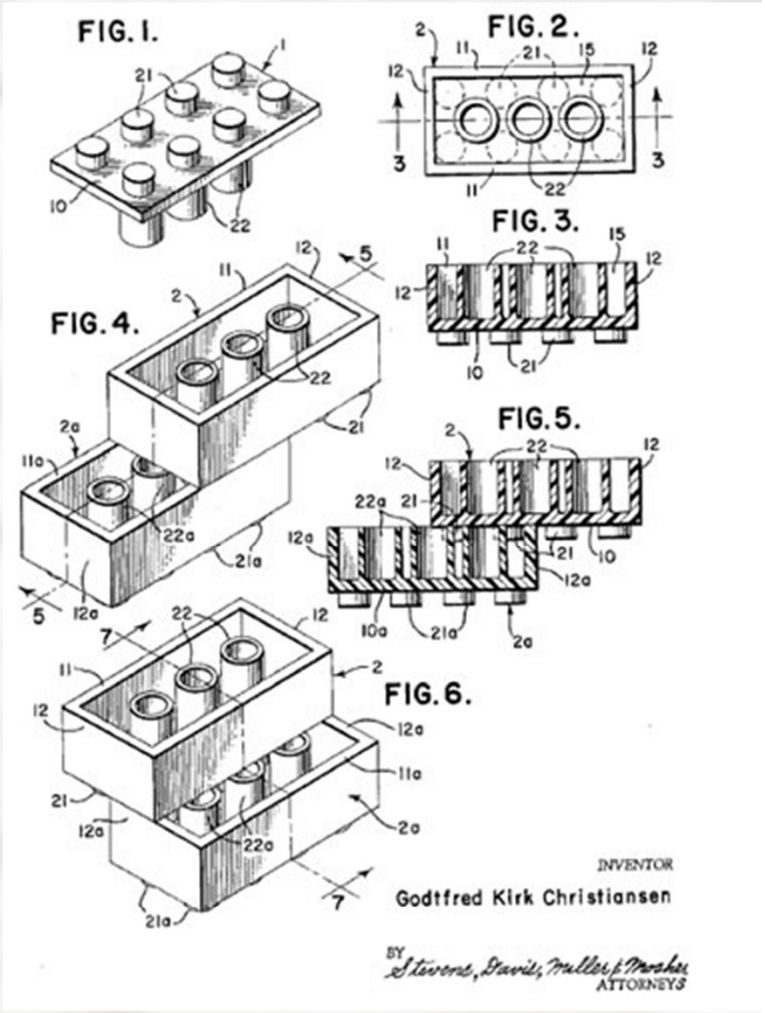
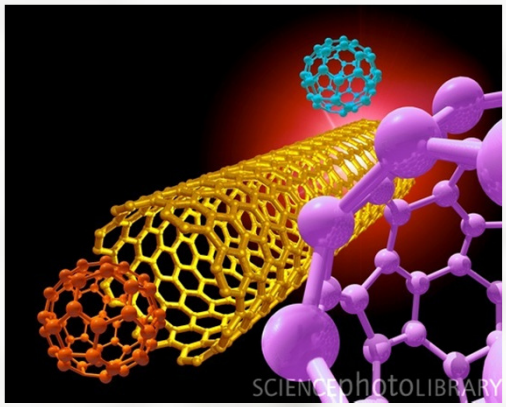


FIG. 1.
FIG. 2.
FIG. 3.
FIG. 4.
FIG. 5.
FIG. 6.
INVENTOR
Godtfred Kirk Christiansen
ATTORNEYS

Computational approaches

- Molecular Modeling
- Quantum-Chemical Approaches
- QSARs: Quantitative Structure-Activity Relationships



Physical Properties

Toxicity

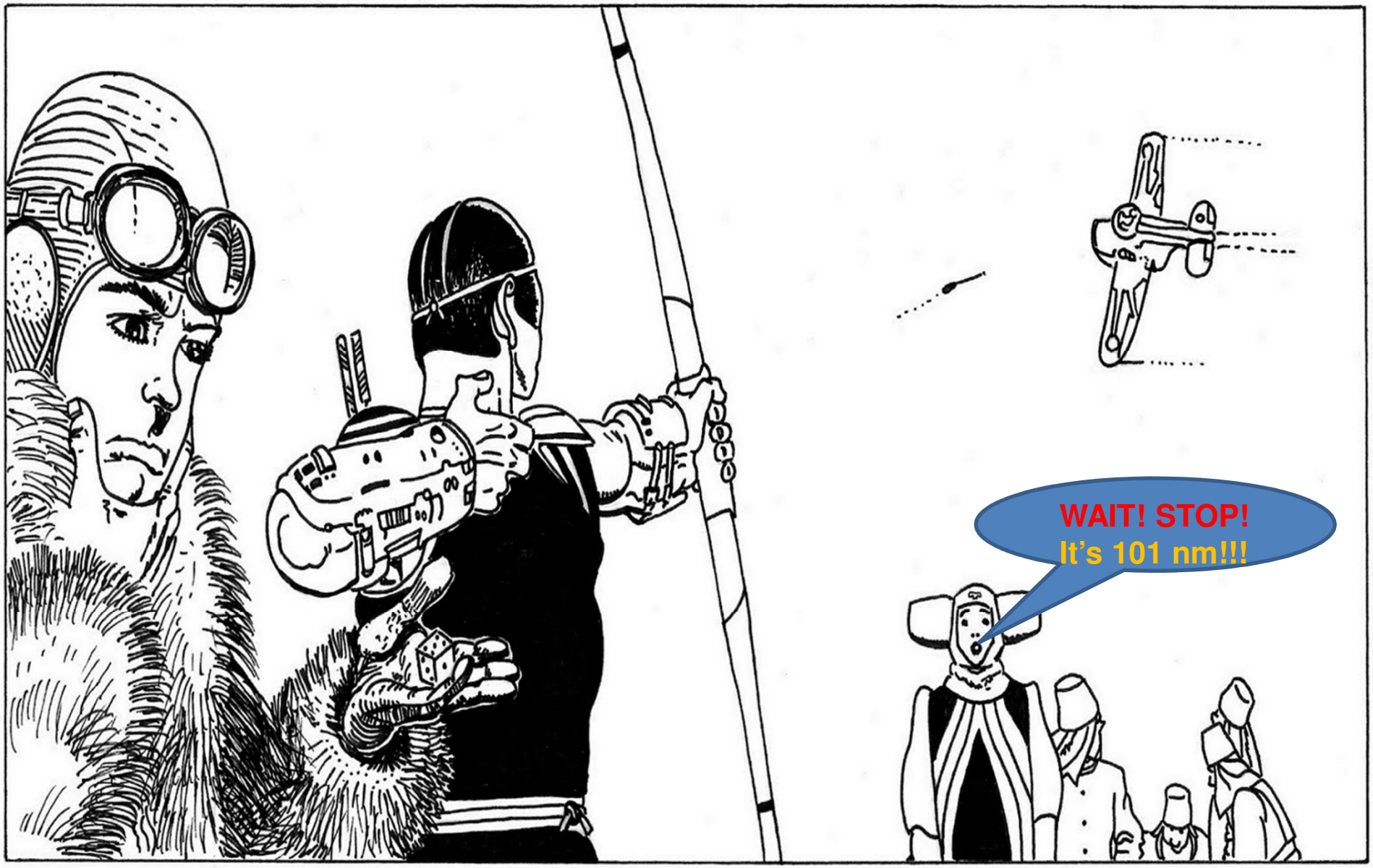
Biokinetic Parameters

Environmental Distribution

The Steps Towards Modeling of Nanoparticles' Properties and Toxicity:

- Development of nanomaterials inventory (database) – collecting the data on experimental physicochemical properties, toxicity endpoints
- Identification of structural descriptors suitable for modeling nanoparticle reactivity
- Modeling the interaction of nanoparticles with biological systems - by means of computational approaches including quantum chemistry methods, molecular modeling and protein-ligand docking techniques
- QSAR modeling - exploring the relationships between structure and properties (for example, solubility), toxicity, using multivariate data analysis techniques

Nano: Is It Just the Size that Matters?



(Q)SAR

=

(Quantitative) Structure-Activity Relationship

IN SILICO

Basic Concept of QSAR Modeling

Endpoint
(experimentally measured)

y

- *Activity (EC_{50})*
- *Phys/Chem property (K_{OW}, $t_{1/2}$)*
- *Retention parameters (t_R)*
- *Toxicity (LD_{50}, LC_{50})*
- *…*

QSAR model

$\mathbf{y} = f(\mathbf{X})$

(*eg.* $\mathbf{y} = b_0 + b_1\mathbf{x_1} + b_2\mathbf{x_2}$)

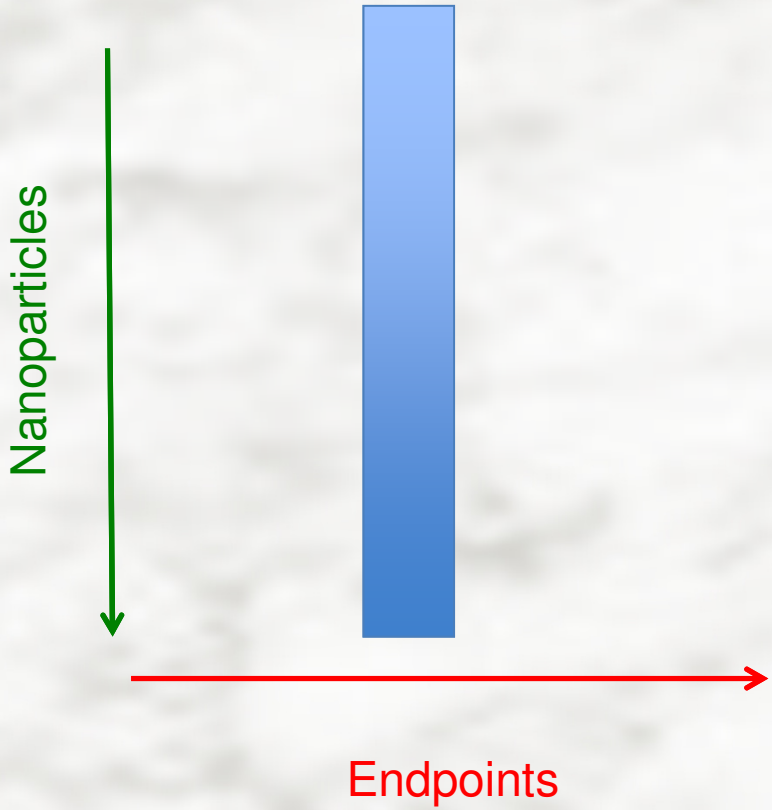
- *Linear Regression (LR)*
- *Multiple Linear Regression (MLR)*
- *Partial Least Squares (PLS)*
- *Artificial Neural Networks (ANN)*
- *…*

Structural descriptors

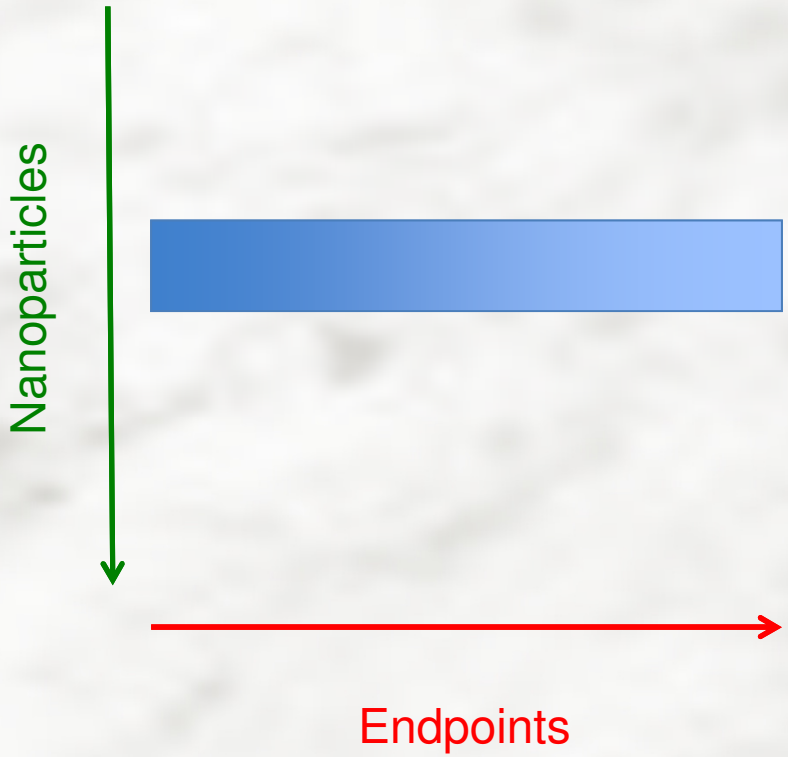
X

- *Dipole moment*
- *Polarizability*
- *HOMO, LUMO*
- *Topological indexes*
- *Number of specific atoms/groups*
- *…*

Experimentalists vs. QSAR modelers



Data for QSAR



Data from experiments

Existing databases

OECD database

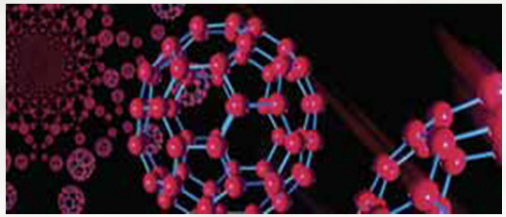
http://webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx?

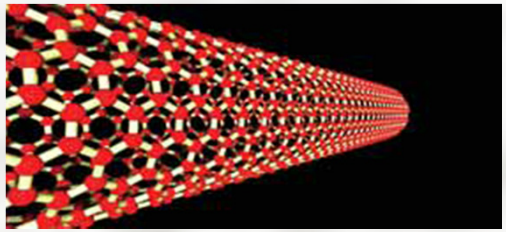
JRC NANOhub database

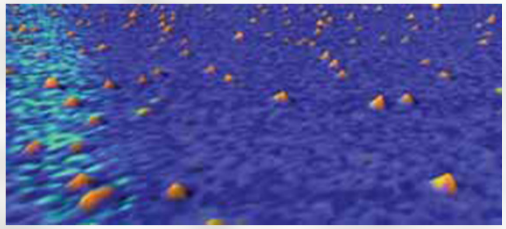
(http://www.napira.eu)

Specific structural features of NPs

Oberdörster et al. *Particle and Fibre Toxicol.* 2: 8.





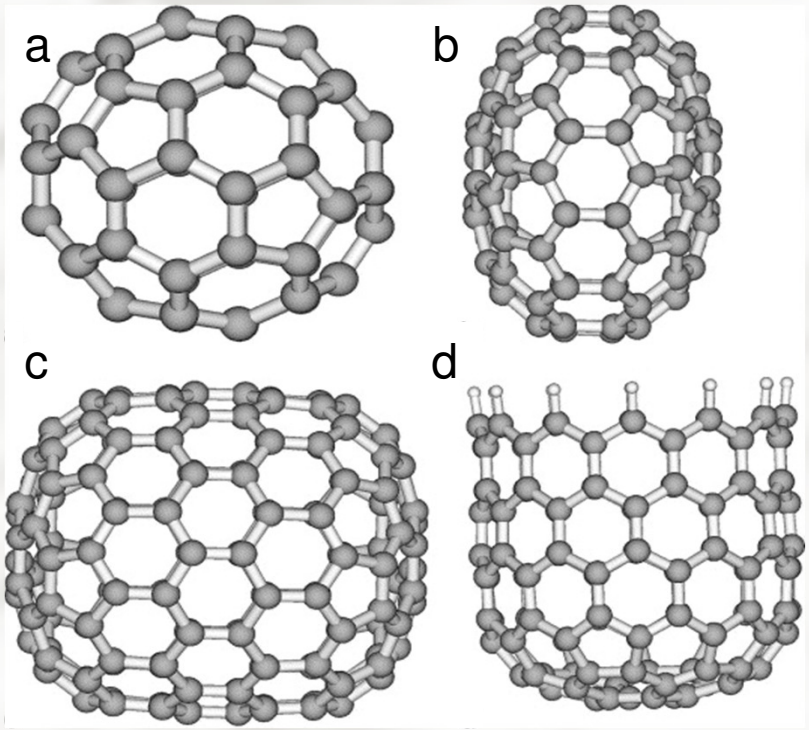


Toxicity of NPs can be related to:

- size
- size distribution
- agglomeration state
- shape
- porosity
- surface area
- chemical composition
- structure-dependent electronic configuration
- surface chemistry
- surface charge
- crystal structure

Calculating 3D descriptors based on the whole system

Shukla, Leszczynski (2006) *Chem. Phys. Lett.* 428: 317-320.



Calculations performed at the Density Functional Theory (DFT) level: B3LYP/6-31G(d)

#	Structure	HOMO-LUMO gap [eV]	IP [eV]	EA [eV]
a	Fullerene C_{60}	2.77	7.24	1.75
b	Disk C_{96}	1.53	6.46	2.98
c	Capsule C_{144}	1.25	6.72	3.46
d	Bowl $C_{120}H_{12}$	0.46	5.19	3.75

Experimental techniques that can be used to obtain nano-descriptors

Haselov et al. (2008) *Ecotoxicology* 17: 344-361.

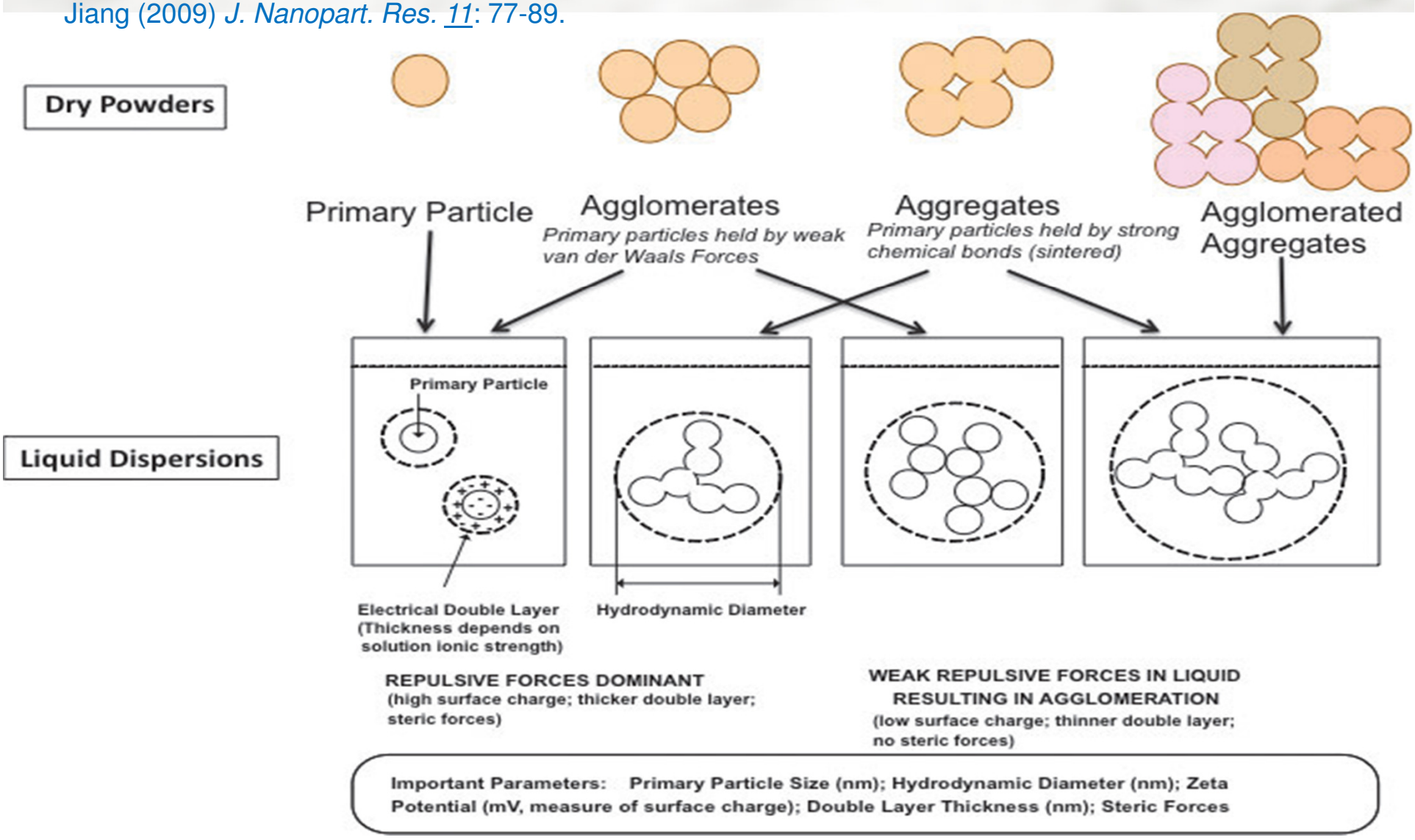
Properties	Instruments and methods*
Diameter	EM, AFM, Flow-FFF, DLS
Volume	Sed-FFF
Area	EM, AFM
Surface charge	z-Potential, electrophoretic mobility
Crystal structure	XRD, TEM-XRD
Elemental composition	Bulk: ICP-MS, ICP-OES Singe nanoparticle: TEM-EDX Particle population: FFF-ICP-MS
Aggregation state	DLS, AFM, ESEM
Hydrophobicity	Liquid-liquid extraction chromatography
Hydrodynamic diameter	Flow-FFF, DLS
Equivalent poresize diameter	Particle filtration

Abbreviations:

- EM- electronic microscopy,
- AFM - atomic force microscopy,
- FFF- field flow filtration,
- DLS - dynamic light scattering,
- LC- liquid chromatography,
- XRD - X-ray diffraction,
- TEM - transmission electron microscopy,
- ICP-MS - inductively coupled plasma mass spectrometry,
- ICP-OES - inductively coupled plasma emission spectroscopy,
- EDX - energy dispersive X-ray spectrometry,
- ESEM - environmental scanning electron microscopy.

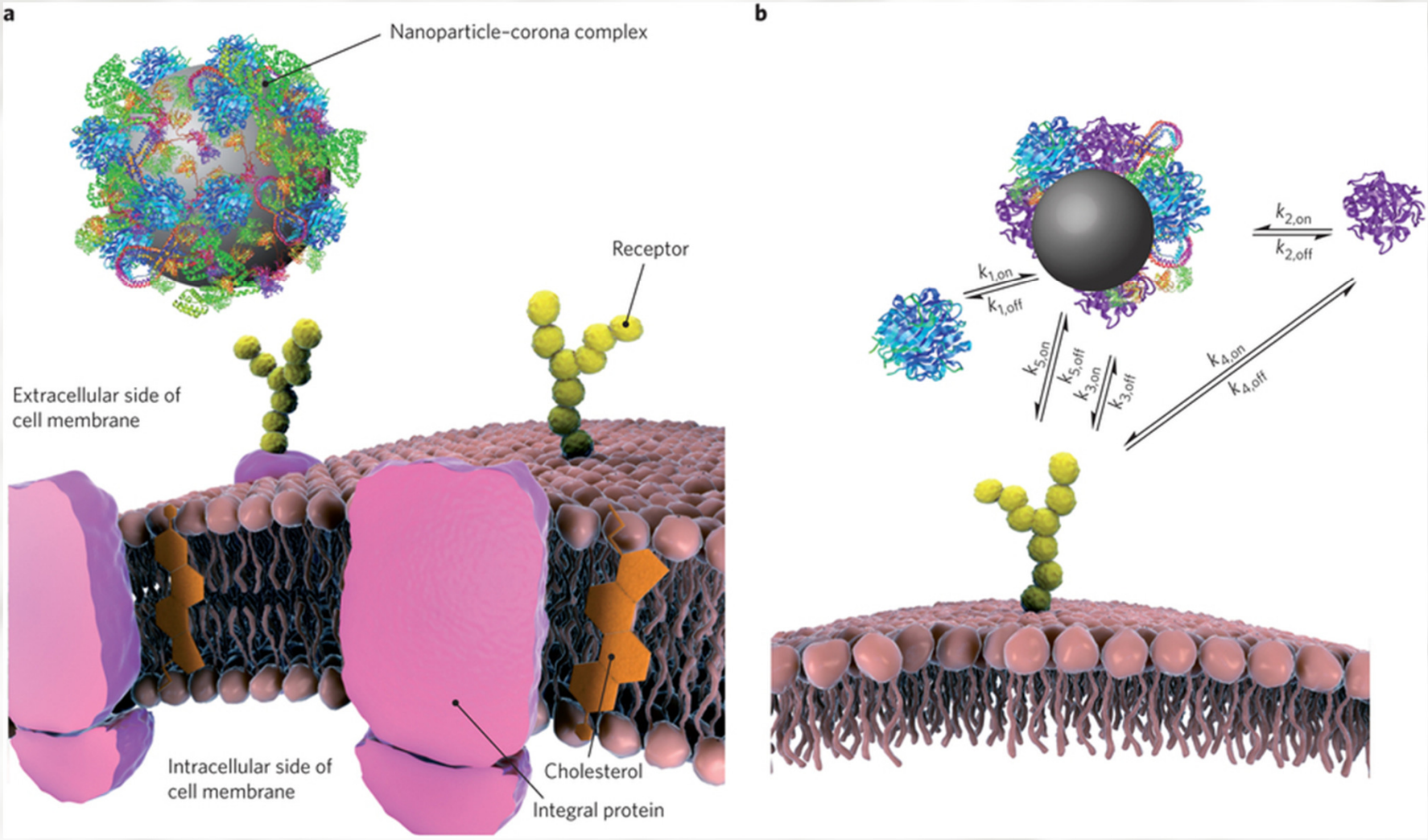
Agglomeration and aggregation of NPs

Jiang (2009) *J. Nanopart. Res.* *11*: 77-89.



Formation of Protein Coronas

Marco Monopoli, Christoffer Åberg, Anna Salvati & Kenneth Dawson, Nature Nanotechnology, 7, 779–786 (2012)



that affect the different modules should be independently optimized to ensure higher accuracy in the predicted output (Fig. 1c). For example, the variability (that is, noise)[8] in the expression level of the synthetic genes between individual cells in a population of cells should be minimal so that responses between them are more consistent. To create larger and more complex networks, such noise should eventually be eliminated because it can propagate in a network and affect the predictability of the individual modules.

One of the key objectives in synthetic biology is to design networks that can function without interfering with the host regulatory system and this can be achieved by constructing systems that are orthogonal (that is, independent)[3,9]. In such a system, there are minimal or no off-target interactions with the host components. In the present case, orthogonality may be implemented by using transcription factors that have been engineered to bind uniquely to the region on the gene where transcription is initiated. This may permit a hierarchy of regulatory cascades to be constructed, where the reporter protein goes on to act as another transcription factor that may eventually regulate the genes of the host to bring about a physiological change.

With the recent publication of a study on the first synthetic prokaryotic genome[10], we foresee that transplantation of large modular synthetic networks into bacterial and complex organisms will soon become a reality. These are definitely exciting times for synthetic biologists. ❐

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5. Leisner, M., Bleris, L., Lohmueller, J., Xie, Z. & Benenson, Y. *Nature Nanotech.* **5,** 666–670 (2010).
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BIONANOSCIENCE

Nano meets bio at the interface

A method that characterizes the adsorption of a set of small molecules on different nanoparticles may offer a way to predict how proteins interact with them.

Jerzy Leszczynski

As nanoparticles are increasingly being used in commercial products it becomes more and more important to understand how they interact with living organisms and the environment. Nanoparticles are known to selectively absorb proteins[1] (to form a coat known as the 'protein corona') and DNA molecules[2], so being able to predict how and which proteins and other biologically important species are adsorbed, and understanding more about these interactions, should help us to reduce any adverse impacts of nanoparticles on human health and the environment.

Writing in *Nature Nanotechnology*, Jim Riviere and colleagues[3] of North Carolina State University report an approach called the biological surface adsorption index (BSAI) to characterize the interaction between different chemical species and nanoparticles, and to use this information to predict the adsorption behaviour of a number of small molecules on materials ranging from carbon nanotubes to metal oxides.

Biological adsorption processes are complex and they involve the nanoparticle surface, the solid–liquid interface and the protein corona–media interface[4]; at each interface different molecular forces are at work. To understand this complex behaviour, Riviere and co-workers have attempted to build up a general set of parameters that describe the adsorption of a diverse set of small molecules onto the surface of nanoparticles by

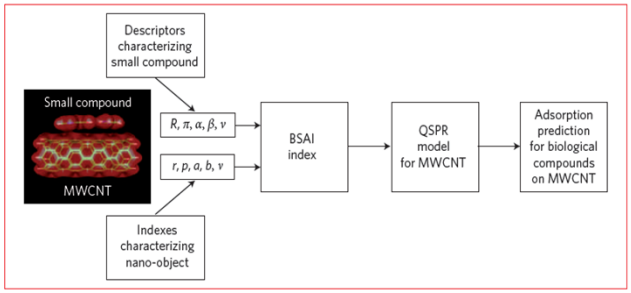
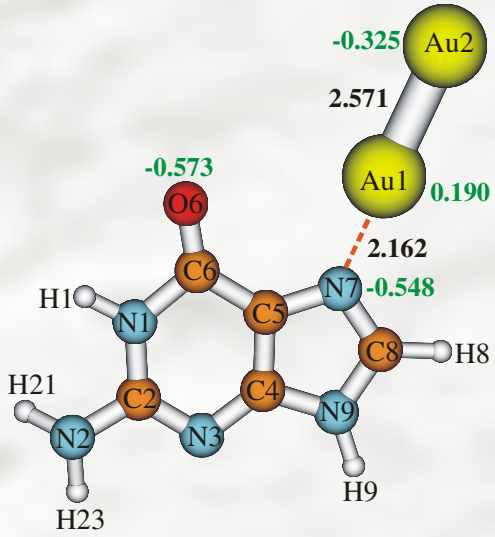


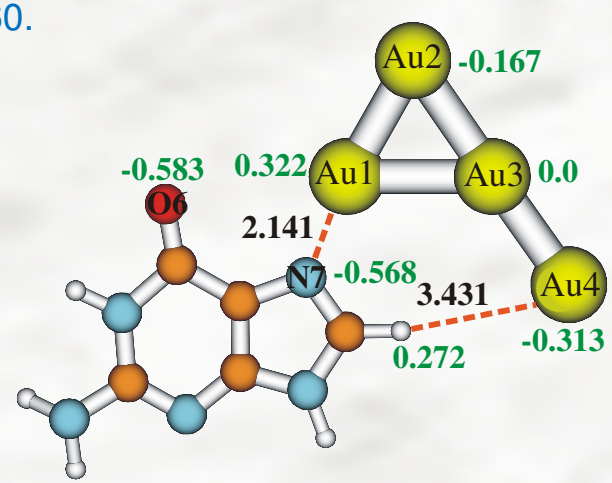
Figure 1 | Development of models for the BSAI approach. BSAI is used to predict the forces involved in the adsorption of small compounds on nanoparticles. The first box shows an example interaction between a multiwalled carbon nanotube (MWCNT) and a guanine–cytosine base pair (depicted by electron density, purple circles) that could be measured and predicted theoretically using BSAI. The experimental measurements are used to develop a set of descriptors (where *R* represents the molecular force from lone-pair electrons, π is polarizability, α is hydrogen bond acidity, β is hydrogen bond basicity, and *v* is London dispersion) that feed into the BSAI model, which contains regression coefficients (*r*, *p*, *a*, *b*, *v*) that represent the relative contributions of the forces. Such descriptors could be used in QSPR modelling (next box) to predict the adsorption of biomolecules on nanoparticles, which might, in turn, be used to develop pharmacokinetics models and assist in their risk assessment.

Back to QM - Optimized Structures of G-Au$_n$ & GC-Au$_n$

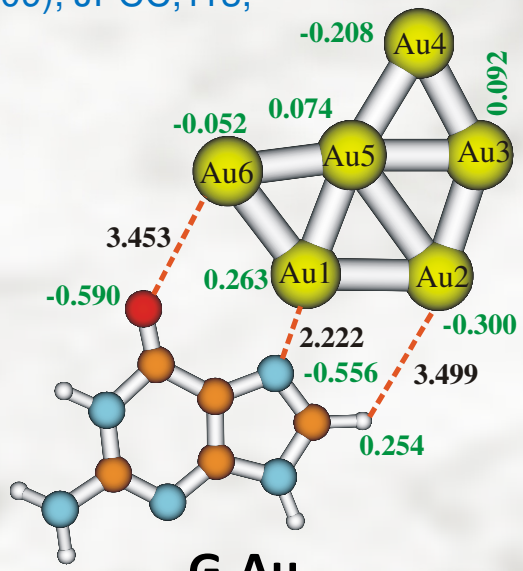
M.Shukla, M.Dubey, E. Zakar J.Leszczynski, (2009), JPCC,113, 3960.



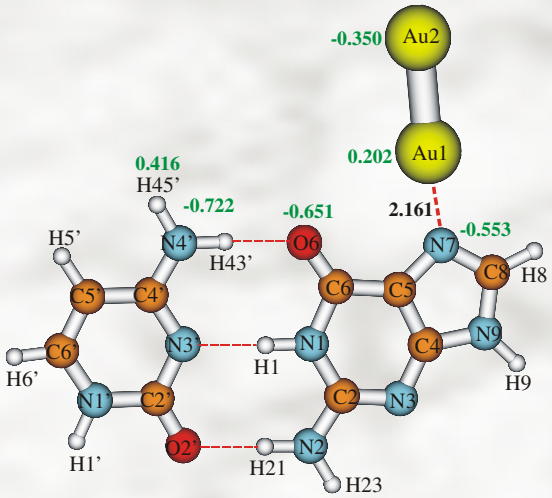
G-Au$_2$



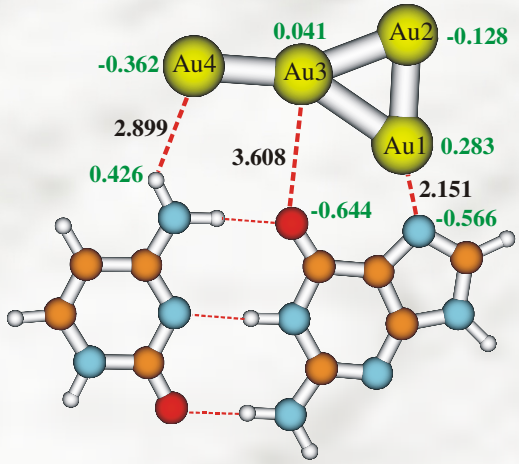
G-Au$_4$



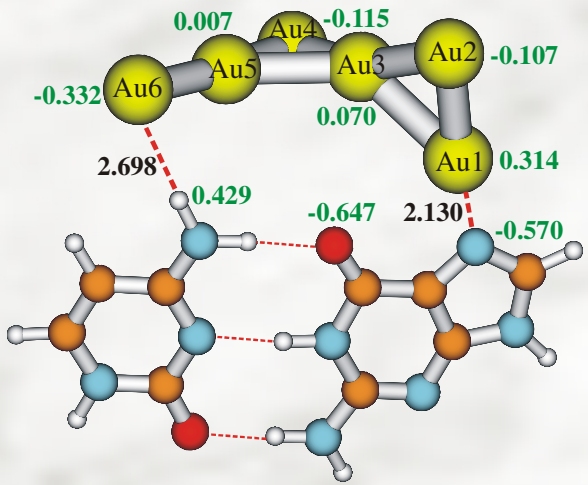
G-Au$_6$



GC-Au$_2$



GC-Au$_4$

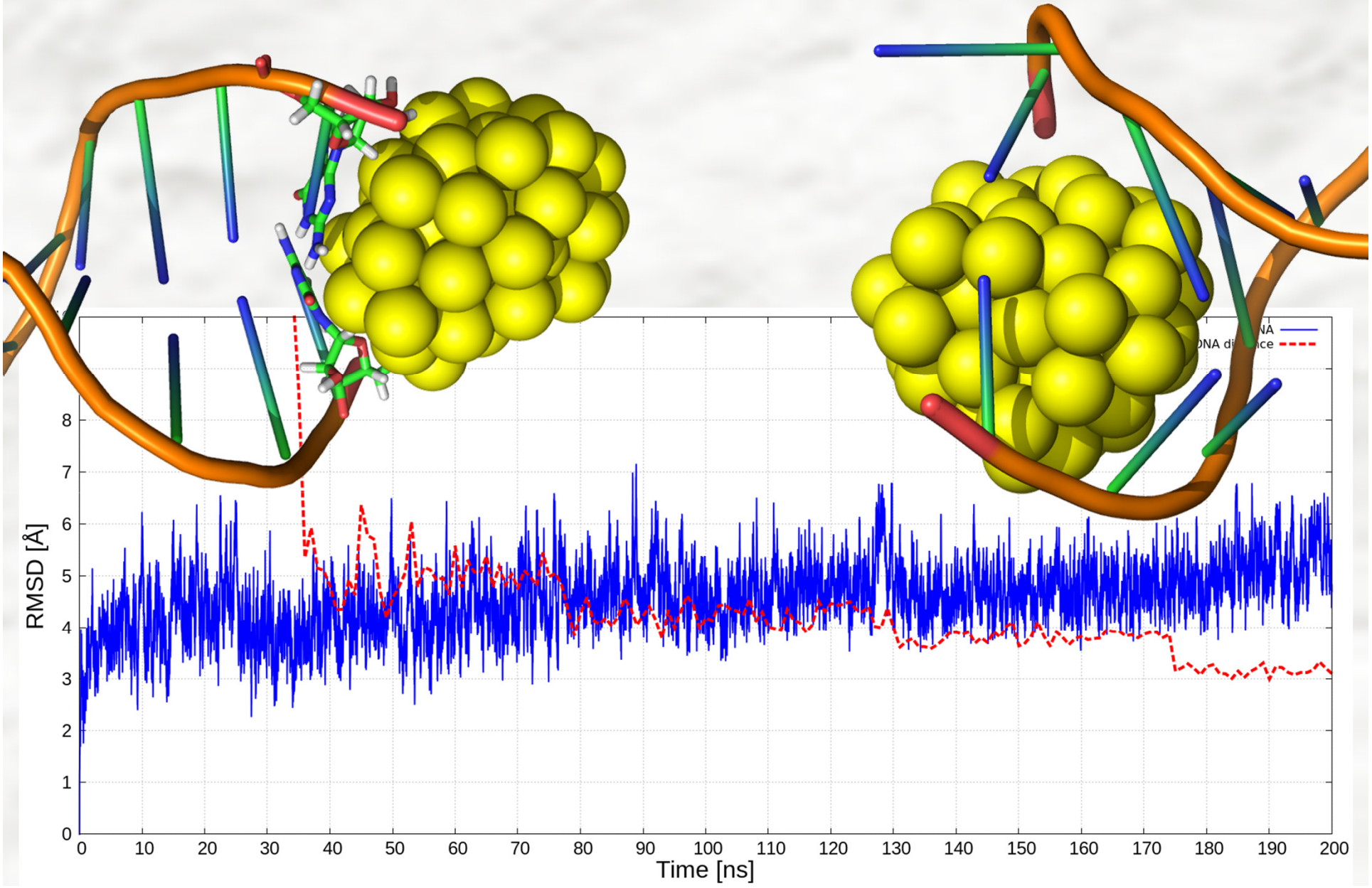


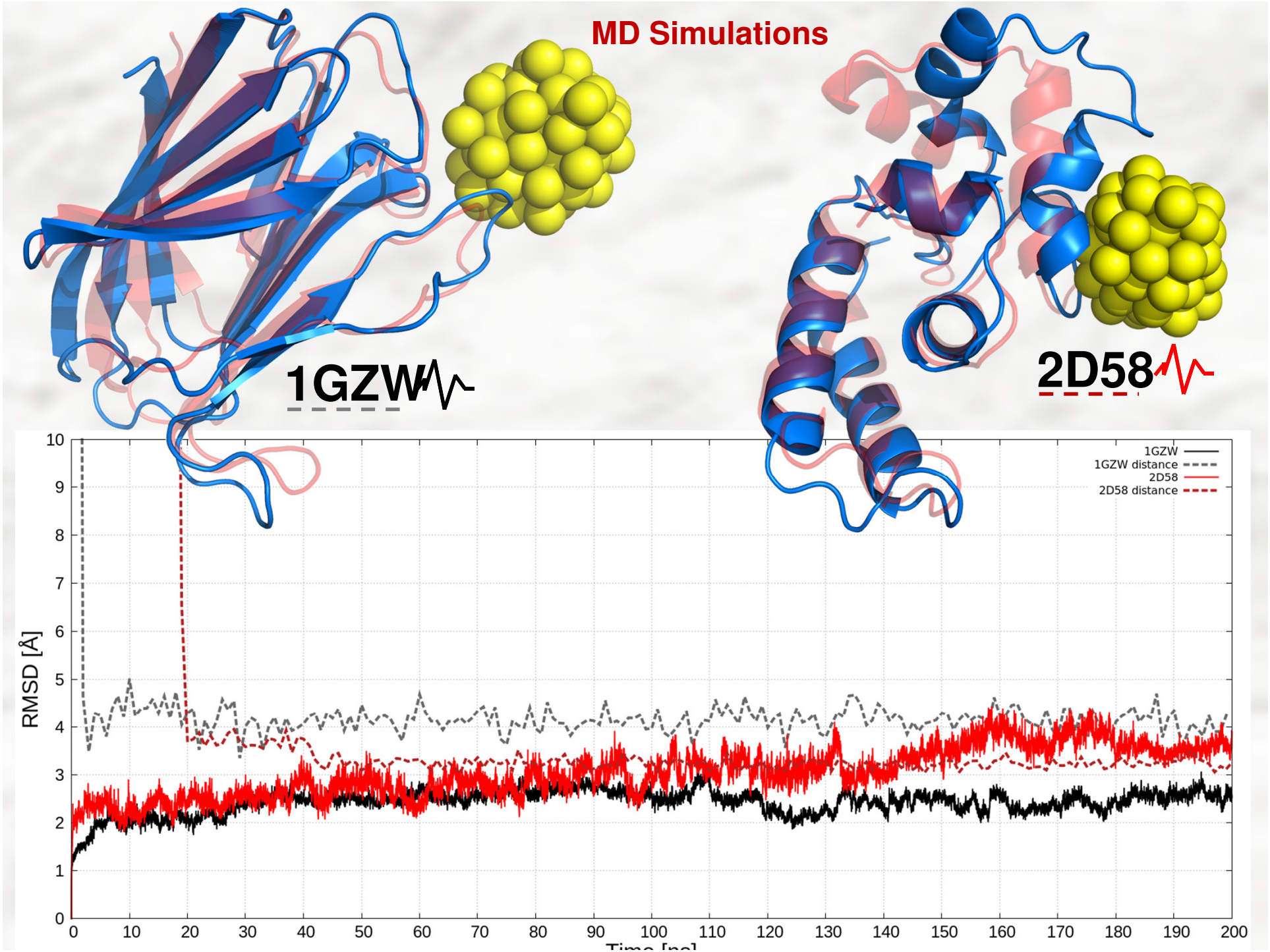
GC-Au$_6$

NBO charges for selected atoms are given in green color while selected gold atoms and base distances are in Å.

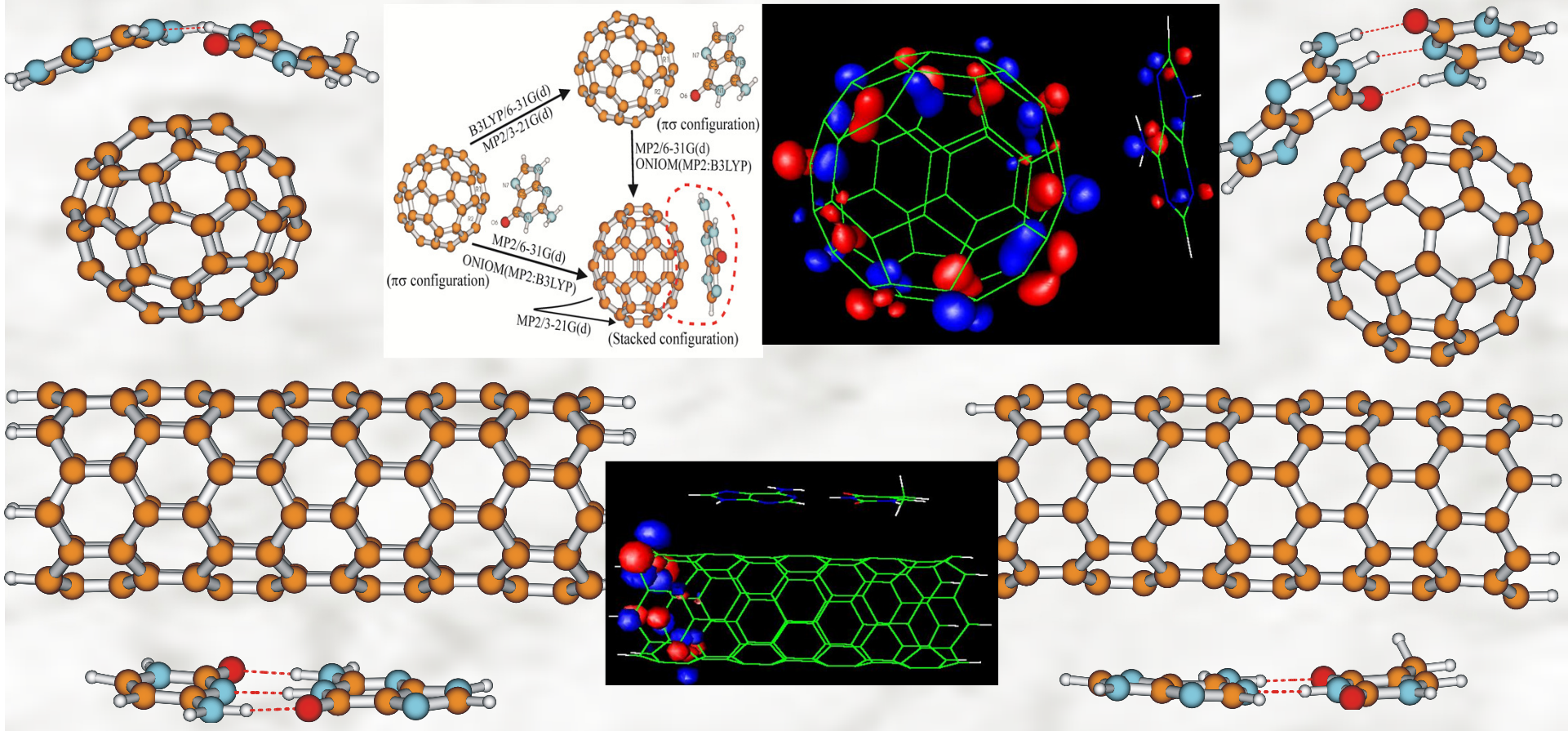
Structure at 35 ns

Structure at 200 ns



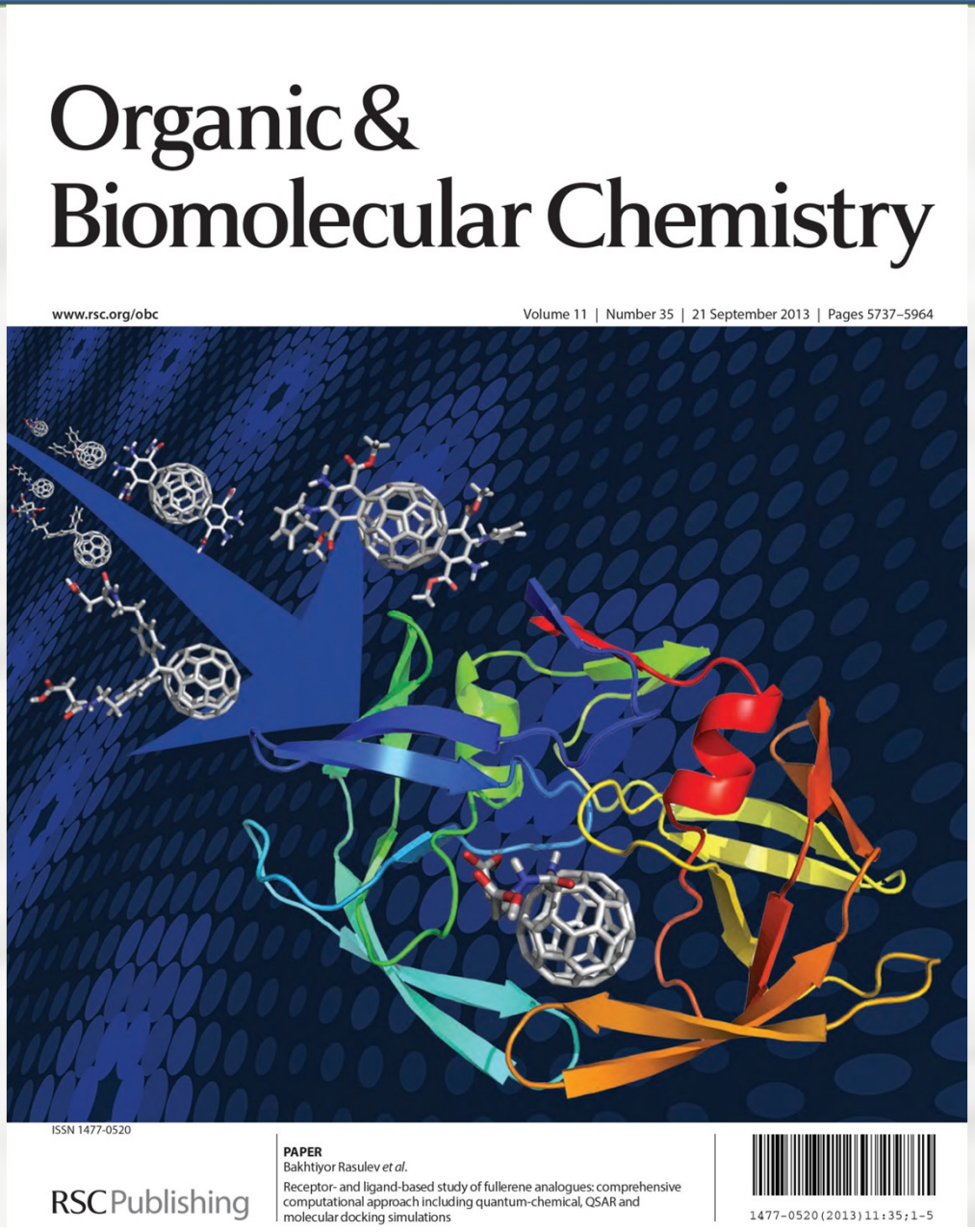

MD Simulations
1GZW
2D58
1GZW
1GZW distance
2D58
2D58 distance
RMSD [Å]
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

Interactions of G, GC and AT with C60 and SWNT

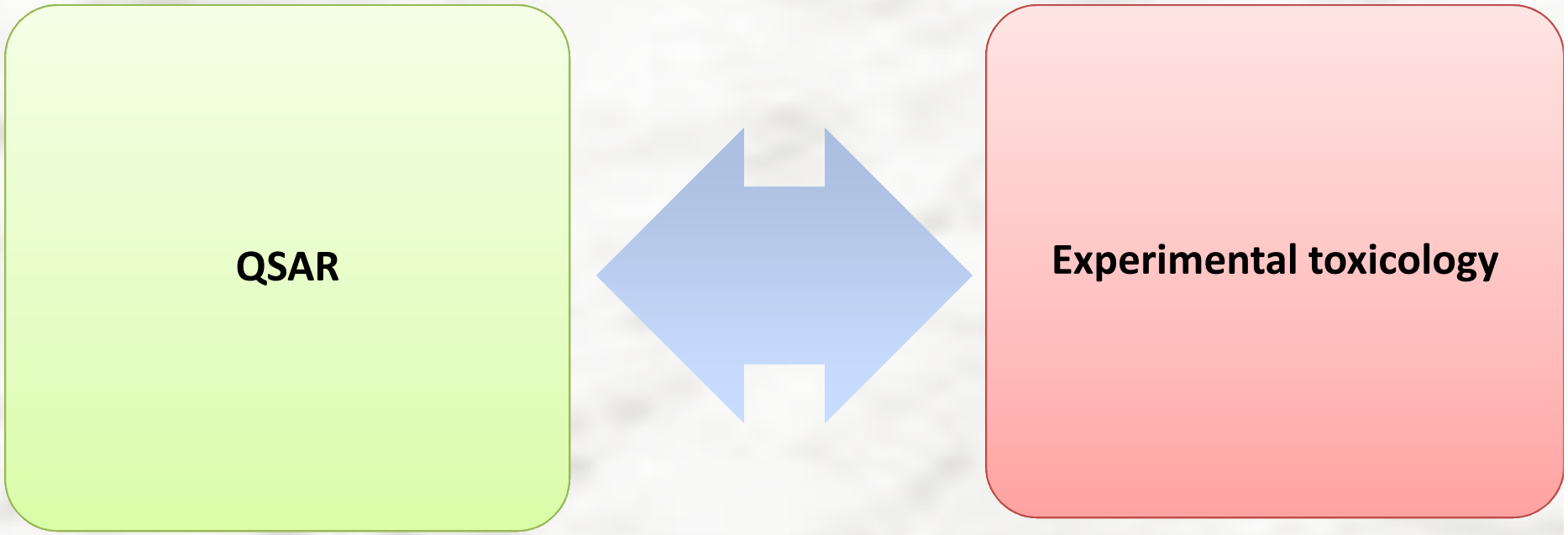


M.Shukla, J.Leszczynski, (2009), CPL, 469, 27; ibid (2010), 493,126; ibid (2010), 496, 130

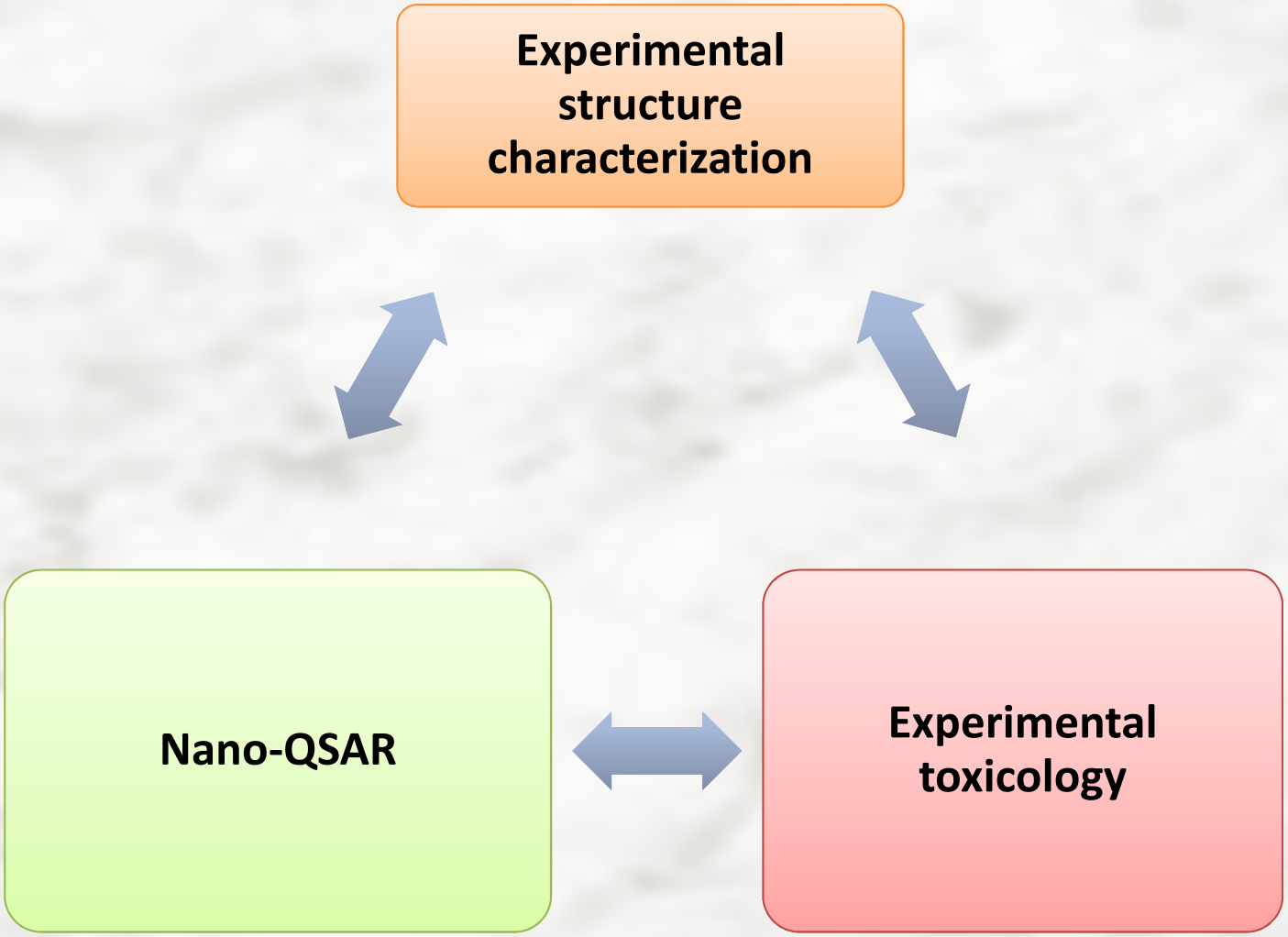
Proteins and Fullerenes



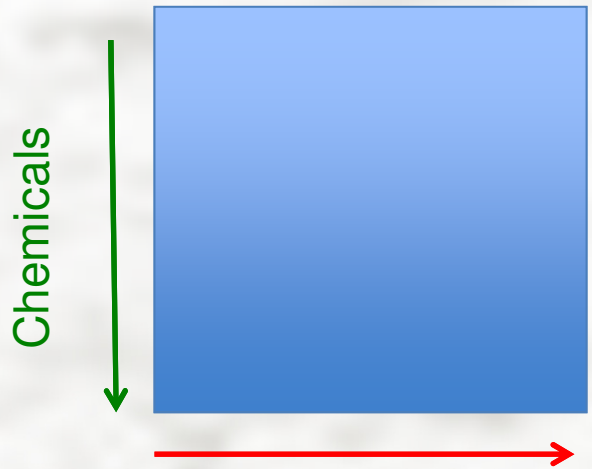
Collaboration Within „Classic" QSAR Studies



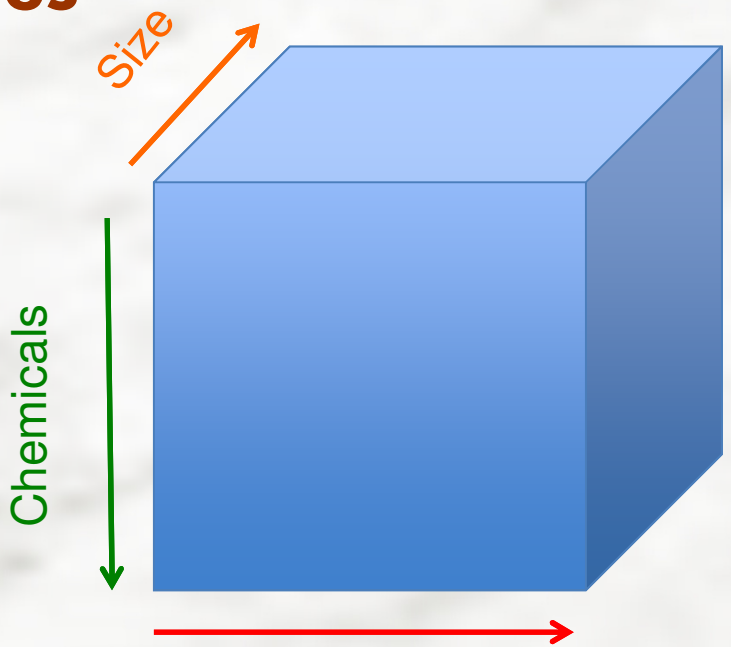
What we Need to Develop Nano-QSARs?



Data for „Classic" QSAR and Nano-QSAR Studies



„Classic" QSAR



Nano-QSAR

Nano-QSAR

Toxicity of Nanomaterials

Nano-QSAR model, which successfully predicted the cytotoxicity of the metal oxide nanoparticles

$$\log(1/EC_{50}) = 2.59 - 0.50 \cdot \Delta H_{Me+}$$

nature nanotechnology
LETTERS
PUBLISHED ONLINE: 13 FEBRUARY 2011 | DOI: 10.1038/NNANO.2011.10

Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles

Tomasz Puzyn[1,2], Bakhtiyor Rasulev[1], Agnieszka Gajewicz[1,2], Xiaoke Hu[3], Thabitha P. Dasari[3], Andrea Michalkova[1], Huey-Min Hwang[3], Andrey Toropov[4], Danuta Leszczynska[5] and Jerzy Leszczynski[1]*

It is expected that the number and variety of engineered nanoparticles will increase rapidly over the next few years[1], and there is a need for new methods to quickly test the potential toxicity of these materials[2]. Because experimental evaluation between the structures of 17 metal oxides and their cytotoxicity to *E. coli* cells. Based on this model and experimental data[6], we have hypothesized the most probable mechanism for the cytotoxicity of these nanoparticles. We investigated this cytotoxicity in bacteria,

Modeling

Experiment

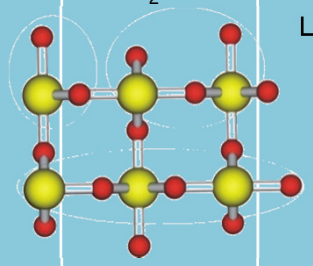
Structural, Physical and Quantum-Chemical Properties

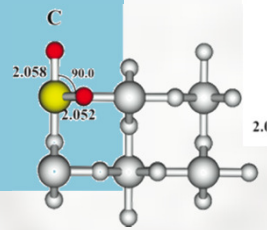
***In vitro* data**

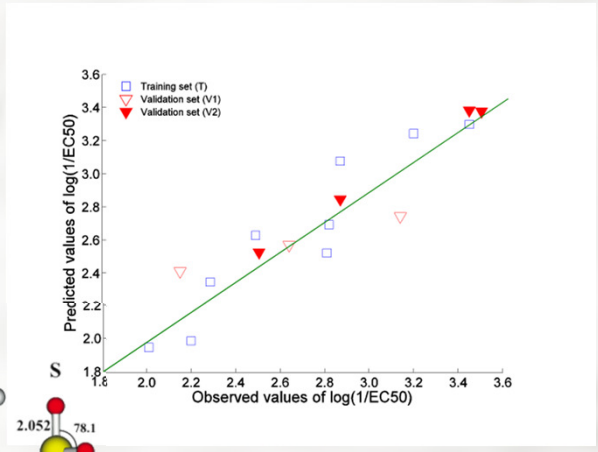
QSAR model

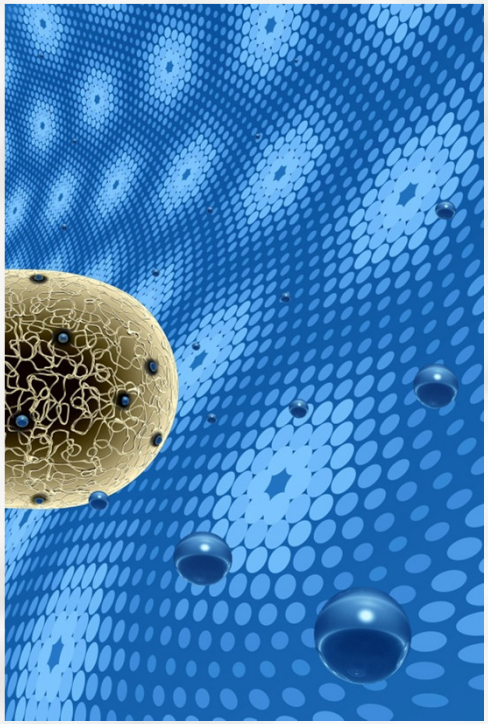
Predicting the activity for untested compounds

Training Set	Validation set 1	Validation Set 2
ZnO	V_2O_3	CoO
CuO	Sb_2O_3	NiO
Y_2O_3	ZrO_2	Cr_2O_3
Bi_2O_3		La_2O_3
In_2O_3		
Al_2O_3		
Fe_2O_3		
SiO_2		
SnO_2		
TiO_2		





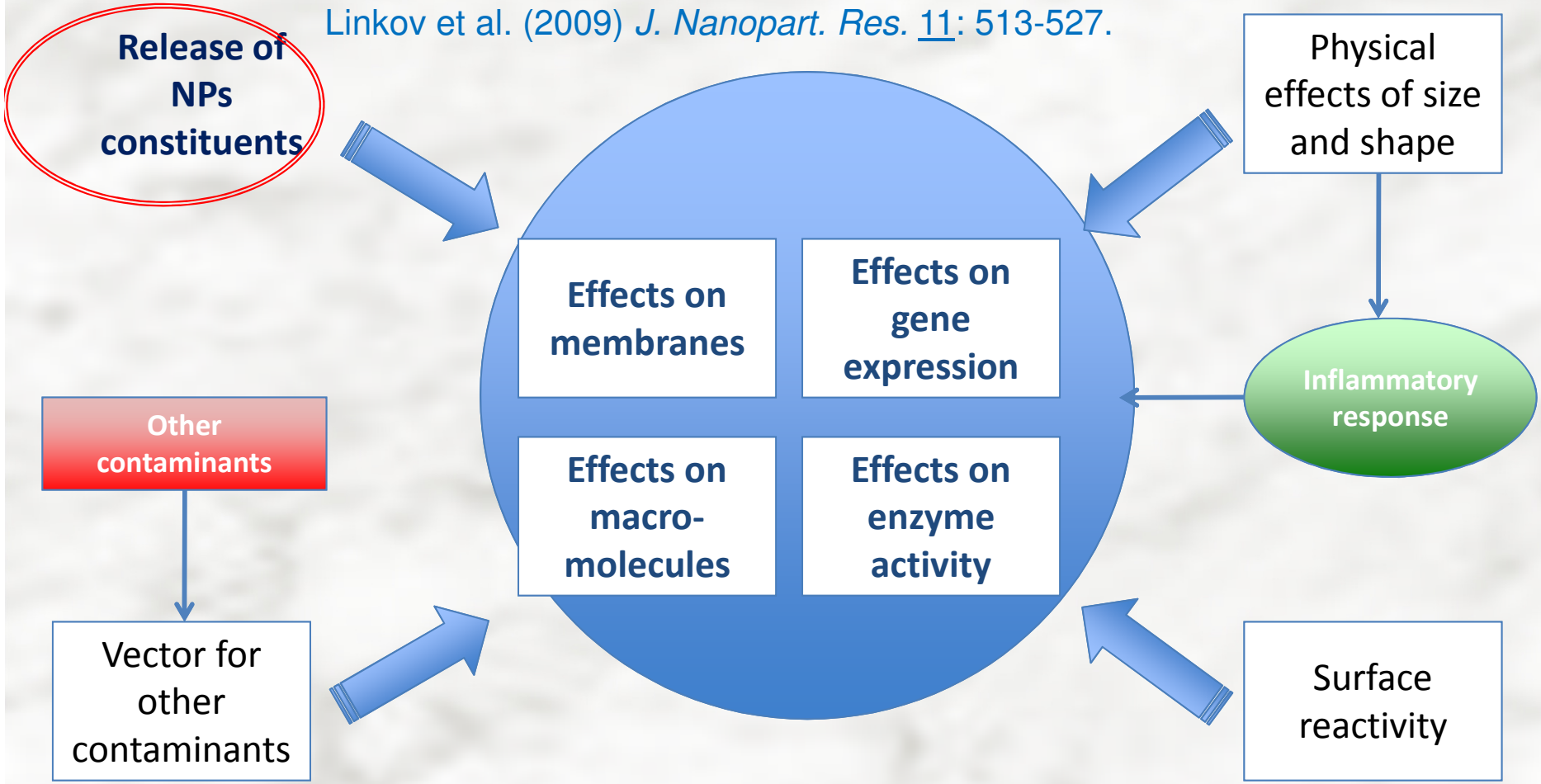


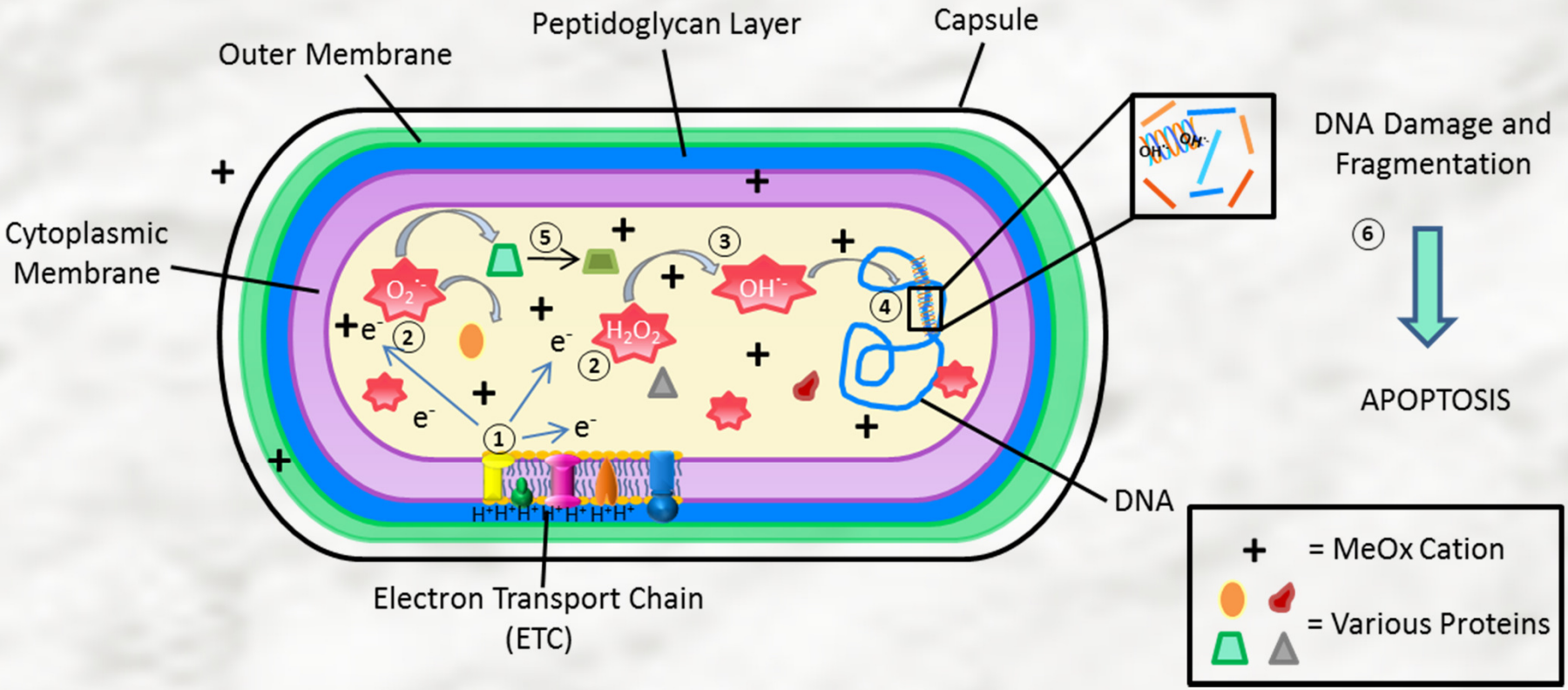


E. coli **and nanoparticle surface**

Postulated mechanisms of NPs' toxicity

Linkov et al. (2009) *J. Nanopart. Res.* 11: 513-527.





Proposed Mechanism of Bacterial Cell Death

1. Electrons leak from ETC during respiration. 2. The free electrons interact with various molecules in the cell to produce free radicals. 3. MeOx cations in the cell increase the production of free radicals, particularly the $OH^{\bullet -}$. 4. The $OH^{\bullet -}$ attacks the DNA, producing single and double strand breaks and blocks replication of DNA. 5. Proteins are attacked by free radicals and oxidized, which impairs their function. This leads to a more inefficient ETC which then produces more free radicals. 6. The vicious cycle continues until there is too much DNA damage in the cell and apoptosis occurs.

Gajewicz A., Schaeublin N., Rasulev B., Hussain S., Leszczynska D., Puzyn T. and Leszczynski J., Towards Understanding Mechanisms Governing Cytotoxicity of Metal Oxides Nanoparticles: Hints from Nano-QSAR Studies, *Nanotoxicology*, 2014, in press

Towards More Complex Systems: HaCaT Human Cell Line

The developed two-variable predictive model for 18 metal nanooxides can be expressed as:

$$\log (EC_{50})^{-1} = 2.466 + 0.244\, \Delta H_f^c + 0.394\, \chi^c$$

where: ΔH_f^c is the enthalpy of formation of metal oxide nanocluster representing fragment of the surface and χ^c - the Mulliken's electronegativity of the cluster.

$F = 44.6,\ p = 1x10^{-4},\ n = 18,\ R^2 = 0.93,\ RMSE_C = 0.12,\ Q^2_{CV} = 0.86,$
$RMSE_{CV} = 0.16,\ Q^2_{Ext} = 0.83,\ RMSE_P = 0.13$

Gajewicz A., Schaeublin N., Rasulev B., Hussain S., Leszczynska D., Puzyn T. and Leszczynski J., Towards Understanding Mechanisms Governing Cytotoxicity of Metal Oxides Nanoparticles: Hints from Nano-QSAR Studies, *Nanotoxicology*, 2014, in press

Summary Table of Characterization Data, Selected Parameters and Computational Results

	Experimental data				Computational data						
Metal Oxide Nanoparticles	Average Particle Size, (nm)	LC_{50} (μg/mL)	Average Particle Size in media (nm)	Zeta Potential (mV)	ΔH_f^c [kcal/mol]	χ^c [eV]	Observed $\log(LC_{50})^{-1}$ [molar]	Set	Predicted $\log(LC_{50})^{-1}$ [molar]	Residuals	Leverages
Titanium IV oxide	42.3	1389	1307.0 ± 313.7	-9.6 ± 0.2	-1492.0	4.91	1.76	T	1.78	-0.02	0.71
Aluminum III oxide	44	1439	372.3 ± 17.9	-20.2 ± 5.2	-600.0	3.44	1.85	V	1.90	-0.05	0.28
Zirconium IV oxide	46.7	1188	661.4 ± 14.4	-8.5 ± 1.1	-638.1	4.95	2.02	T	2.25	-0.23	0.13
Iron III oxide	32	1483	297.6 ± 6.9	-18.1 ± 7.4	-378.5	4.21	2.05	V	2.21	-0.16	0.17
Silicon IV dioxide	15	453	809.7 ± 97.4	-8.1 ± 1.0	-618.3	3.81	2.12	T	1.99	0.13	0.23
Yttrium III oxide	38	1390	1222.9 ± 351.7	-10.7 ± 1.5	-135.3	3.35	2.21	V	2.14	0.07	0.33
Vanadium III oxide	NIA	855	433.9 ± 40.1	-22.8 ± 0	-139.5	3.24	2.24	T	2.11	0.13	0.35
Chromium III oxide	60	755	616.8 ± 118	5.7 ± 3.6	-235.3	4.36	2.30	V	2.33	-0.03	0.17
Antimony III oxide	90-210	1429	640.3 ± 77. 9	-13.3 ± 0.8	-206.7	4.46	2.31	T	2.37	-0.06	0.17
Nickel II oxide	20	242	223.5 ± 33.2	-12.1 ± 2.1	68.0	4.47	2.49	V	2.52	-0.03	0.29
Bismuth III oxide	90	1489	2029 ± 150.7	-2.3 ± 1.8	-148.5	5.34	2.50	T	2.62	-0.12	0.16
Tungsten VI oxide	30-70	634	179.6 ± 63.2	-9.1 ± 2.0	-715.4	6.73	2.56	V	2.65	-0.09	0.20
Manganese III oxide	29.8	362	291.1 ± 7.5	-3 ± 2.3	-96.3	5.00	2.64	T	2.56	0.08	0.18
Tin IV oxide	46.1	322	264.9 ± 64.9	-10.5 ± 1.1	-266.6	4.57	2.67	V	2.36	0.31	0.15
Cobalt II oxide	<100	110	257 ± 11.9	-3.4 ± 1.1	-786.8	7.44	2.83	T	2.78	0.05	0.32
Lanthanum III oxide	45.6	443	672.9 ± 79.1	-12.8 ± 1.3	-157.7	6.45	2.87	V	2.88	-0.01	0.20
Indium III oxide	29.8	332	224.3 ± 63.1	-9.6 ± 1.5	-52.1	6.78	2.92	T	3.02	-0.10	0.28
Zinc II oxide	71	39	188.9 ± 37.2	-10.8 ± 1.5	-449.4	8.33	3.32	T	3.18	0.14	0.46
Copper II oxide	-	-	-	-	-76.32	4.25	-	P	2.39	-	0.24
Iron II oxide	-	-	-	-	-883.2	5.88	-	P	2.35	-	0.20
Gadolinium III oxide	-	-	-	-	-234.07	5.91	-	P	2.71	-	0.14
Lead II oxide	-	-	-	-	-306.31	5.12	-	P	2.48	-	0.12
Lead IV oxide	-	-	-	-	-269.54	6.13	-	P	2.74	-	0.14

Gajewicz A., Schaeublin N., Rasulev B., Hussain S., Leszczynska D., Puzyn T. and Leszczynski J., Towards Understanding Mechanisms Governing Cytotoxicity of Metal Oxides Nanoparticles: Hints from Nano-QSAR Studies, *Nanotoxicology*, 2014, in press

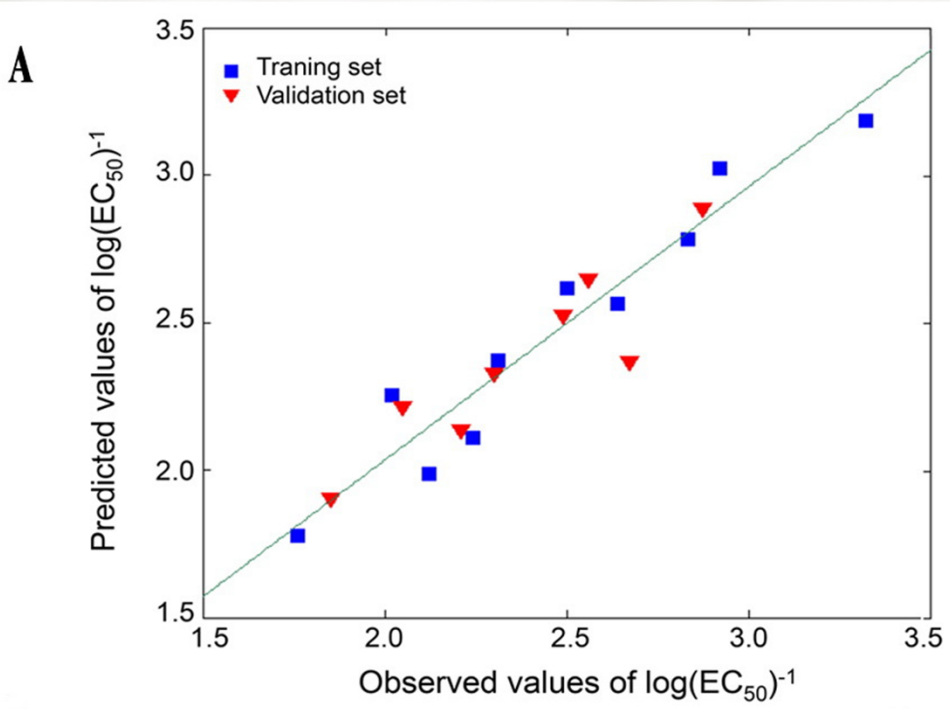


Figure 2. A.
Plot of experimentally determined (observed) versus predicted
log values of $1/EC_{50}$.
The straight line represents perfect agreement between experimental and calculated values. Squares represent values
predicted for the metal oxides from the training set; triangles represent data calculated for metal oxides from the validation sets.
The distance of each symbol from the green line corresponds
to its deviation from the related experimental value.

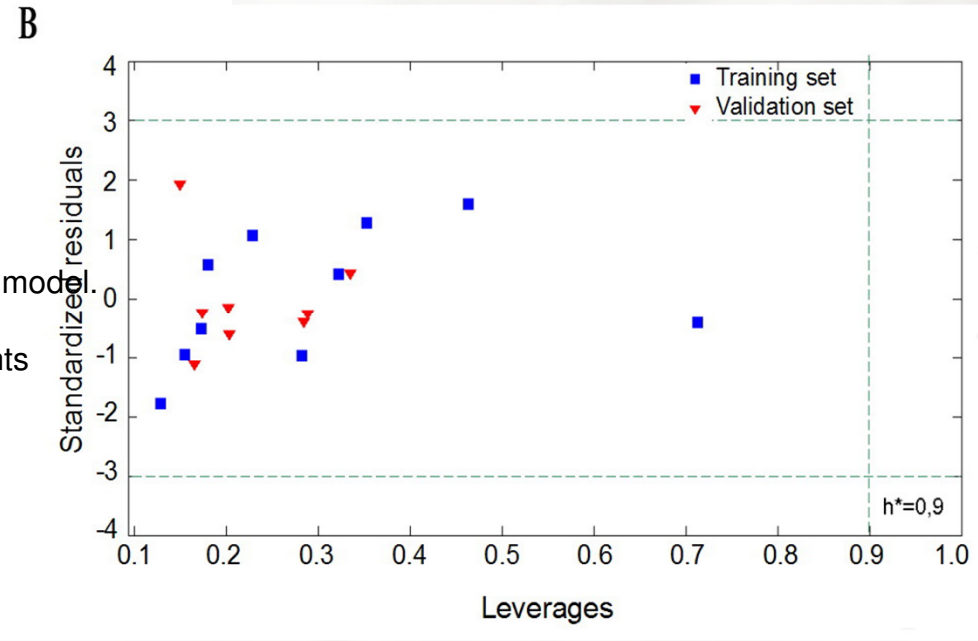


Figure 2. B.
Williams plot describing applicability domains of GA-MLR model. Solid lines represent the residual threshold
(0 ± 3 standard deviation units), and dashed line represents the critical leverage value (h*).

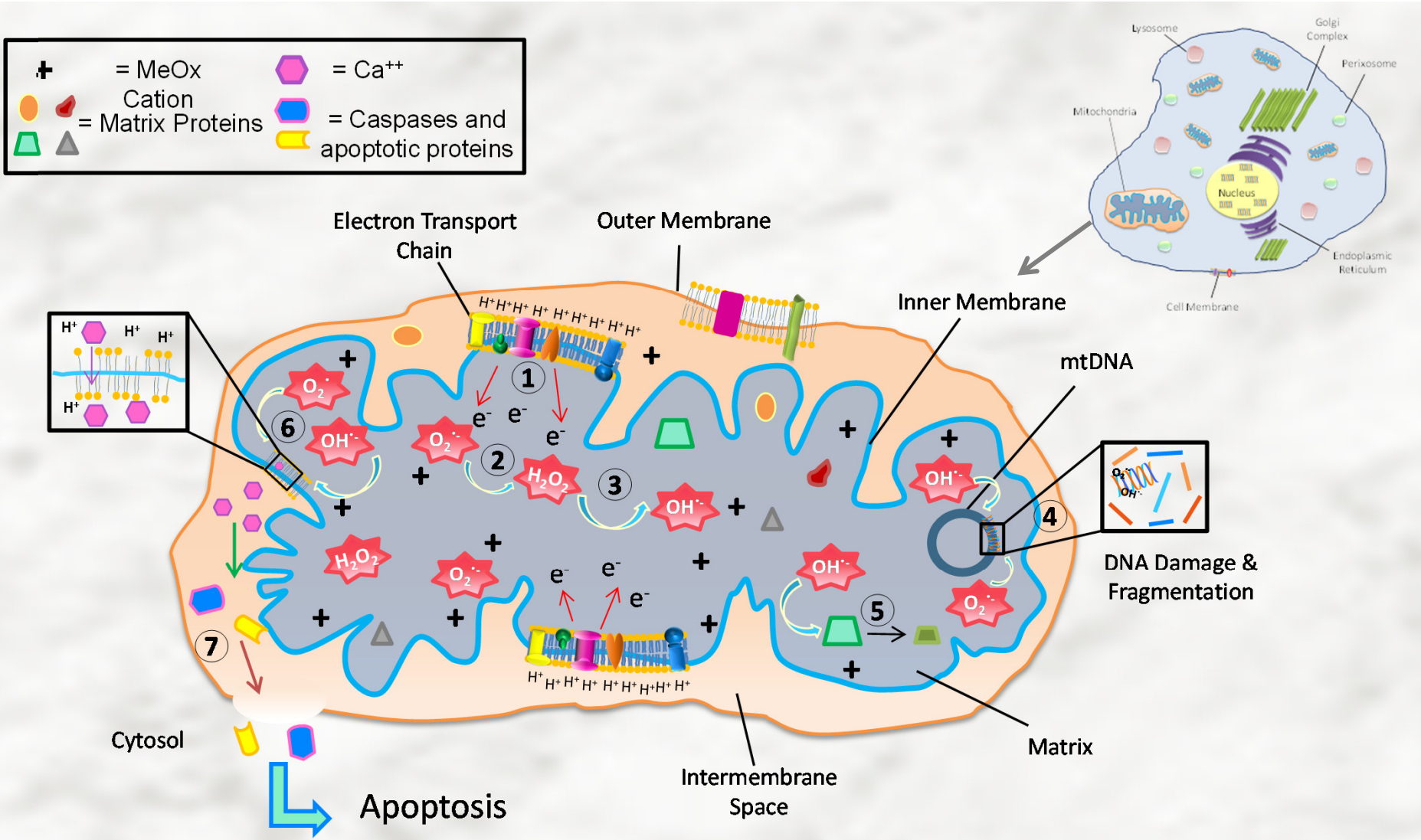
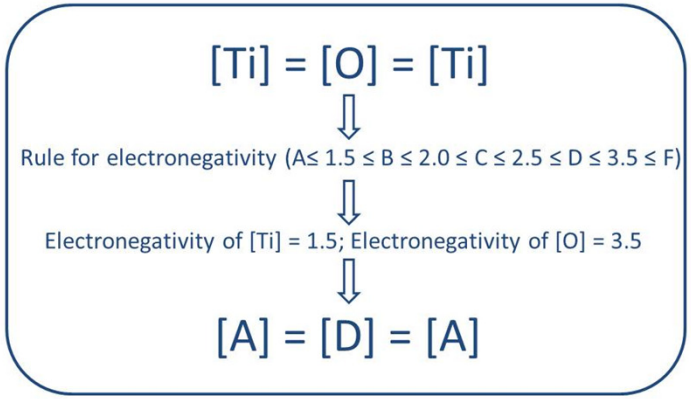


Figure 5. Proposed Mechanism of Eukaryotic Cell Death

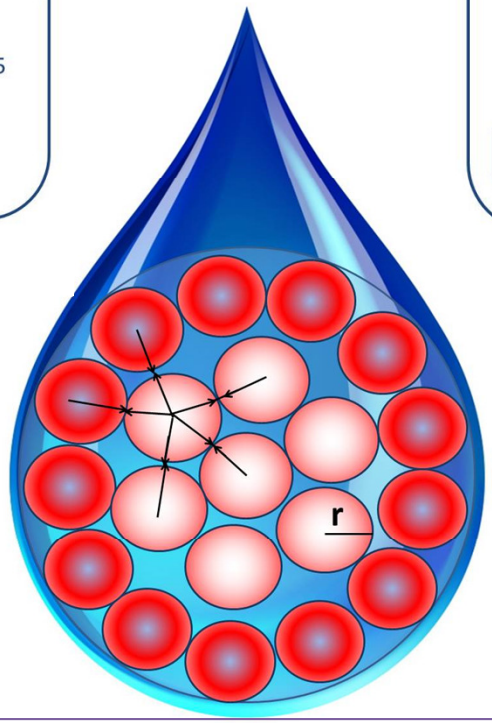
1. Electrons leak from ETC during respiration. 2. The free electrons interact with various molecules in the cell to produce free radicals. 3. MeOx cations in the cell increase the production of free radicals, particularly the $OH^{\bullet -}$. 4. The $OH^{\bullet -}$ and $O_2^{\bullet -}$ attack the mtDNA, producing single and double strand breaks and blocking replication of DNA. 5. Proteins are attacked by free radicals and oxidized, which impairs their function. This leads to a more inefficient ETC which then produces more free radicals. 6. The protonated form of $O_2^{\bullet}$, $OH^{\bullet -}$, and MeOx cause autocatalytic lipid peroxidation. This decreases the fluidity of the membrane which leads to a loss of mitochondrial membrane potential and causes the contents of the matrix to spill into the inner membrane. 7. The release of Ca^{++} and other proteins activates caspases and turns on the mitochondrial apoptosis pathway.

Nano-QSAR Based on SiRMS and "Liquid Drop Model" Techniques

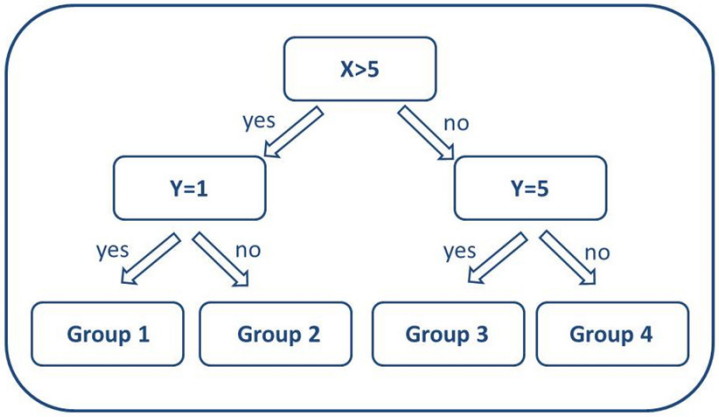
E.Coli and *HaCaT* cells



SiRMS – Simplex Representation of Molecular Structure



"Liquid Drop Model" descriptor representation

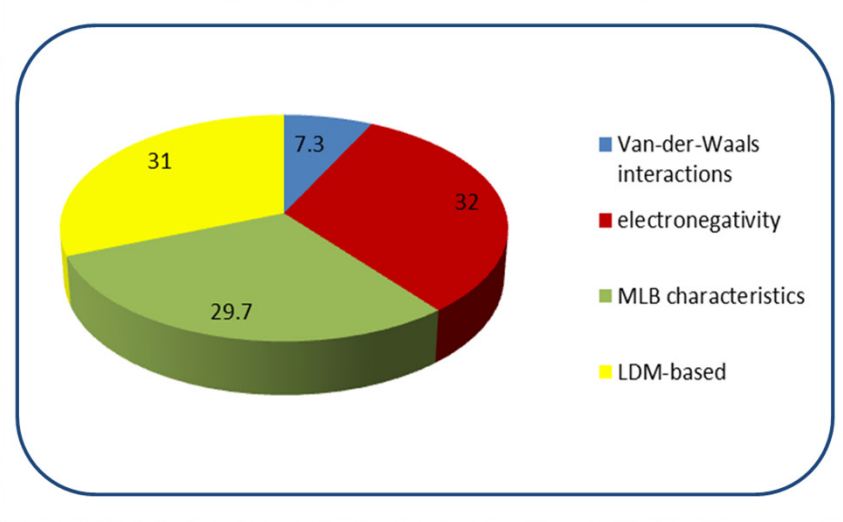


Classification modeling

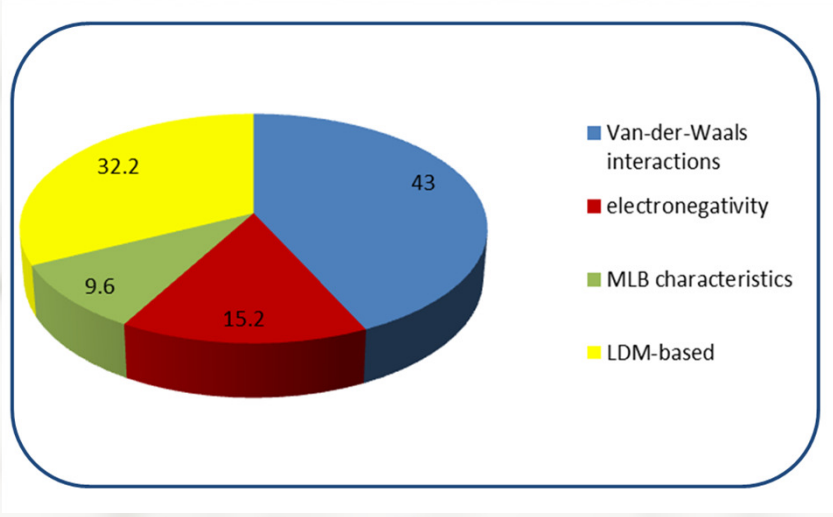
Novoselska N., Rasulev B., Gajewicz A., Kuz'min V., Puzyn T., Leszczynski J. Predictive Classification Models for *HaCaT* and *E.coli* Cells Toxicity of Metal Oxide Nanoparticles, Nanoscale, 6, 13986, **2014**.

Nano-QSAR Based on SiRMS and "Liquid Drop Model" Techniques

Diagram of relative contribution (%) of certain descriptors to toxicity



E.Coli cell toxicity



HaCaT cells toxicity

Novoselska N., Rasulev B., Gajewicz A., Kuz'min V., Puzyn T., Leszczynski J. Predictive Classification Models for *HaCaT* and *E.coli* Cells Toxicity of Metal Oxide Nanoparticles, Nanoscale, 6, 13986, **2014.**

The dose makes the poison

The detailed characterization of the materials is essential in all areas of nanotoxicology.

Fish, worms, rodents, algae, bacteria and cells. Carbon nanotubes, metal oxides and quantum dots. Choose a model system from the first list and a nanomaterial from the second, and chances are that you will be able to find two or more toxicology studies that report slightly different conclusions about the impact of the latter on the former. Twenty years of research has confirmed that nanoscale materials can display unexpected and unusual toxicity, but just how much have we learnt about the interactions between engineered nanomaterials and humans, animals and the environment?

The Society of Toxicology defines toxicology as "the study of the adverse effects of chemical, physical and biological agents on people, animals and the environment"[1], and the sheer diversity of nanotoxicology can be seen on a web page that contains links to all the articles that *Nature Nanotechnology* has published on the subject[2]. One characteristic of nanotoxicology is that materials that are not harmful in their bulk form may well be toxic on the nanoscale. Bulk gold, for example, is normally inert but gold nanoparticles are anything but inert, which is why they are useful for applications such as medical imaging and drug delivery. However, nanoparticles are also more likely to react with cells and various biological components such as proteins, and to travel through organisms, which increases their chances of entering various organs and activating inflammatory and immunological responses[3].

In a typical toxicity test, cells or organisms are subjected to a dose of chemicals, and the response is measured over a period of time; the dose–response relationships obtained in these experiments are important because they are used for determining appropriate dosages for drugs and acceptable limits for exposure to pollutants. However, unlike the soluble chemicals tested in traditional toxicology studies, nanoparticles have shapes and surface areas, and they can diffuse, aggregate/agglomerate and sediment according to their size, density and physical and chemical properties in solution. This means that traditional *in vitro* assays may misrepresent the response and cellular-uptake data for nanoparticles, making the test results less comparable across particle types than for soluble chemicals[4]. On page 385 of this issue Xia and co-workers show that sedimentation of nanoparticles can influence how many nanoparticles are taken up by cells in an *in vitro* assay, and on page 332, Lison and Huaux discuss the different options for defining the relevant cellular dose for such tests.

There are opportunities for computational scientists to work with toxicologists to design new assays.

Another issue in nanotoxicology is the impact of nanomaterials on the environment. Many toxicity studies, until now, have been done at much higher doses than is realistic[3] and they may exemplify Paracelsus's observation of "the dose makes the poison" — toxic substances are harmless in small doses and harmless substances are poisonous when over-consumed. Quantifying real-life occupational exposures and emissions of nanoparticles into the environment is a challenge; modelling studies that consider various release scenarios based on the life cycle of the nanomaterials and products that contain them have been presented, but to improve these models we require data on the industrial production of nanomaterials, the amounts released at different stages of the life cycle of the materials, and the form in which they are released[5].

The chemical and physical properties of nanoparticles have a strong influence on the way in which they interact with biological components or the environment at large, and also on the way they move, accumulate and clear in the body. For example, nanoparticles acquire a 'corona' of proteins when exposed to biological fluids, and this layer is thought to influence the way the cell 'sees' the nanoparticle[6]. It has also been shown that certain nanoparticles can induce proteins to unfold, leading to an inflammatory response[7]. Similarly, nanoparticles are coated with natural organic matter when they enter water, soil or sediment environments and this layer influences their reactivity, bioavailability and other transformations in the environment[8]. These dynamic interactions add complexity to the challenge of determining the biological outcome of nanoparticles.

Studying the influence of the various properties of nanomaterials, the dose, the exposure route and time, and identifying the right model systems is expensive and time consuming. High-throughput and computational approaches are on the horizon to rapidly screen and prioritize nanomaterials for toxicological tests and to develop causal relationships between material properties and biological behaviours[9]. Researchers have shown, for example, that the quantitative structure–activity relationship (a statistical model traditionally applied to chemicals) can predict the cytotoxicity of a small set of metal oxide nanoparticles[10]; there are also opportunities for computational scientists to develop appropriate structural parameters for describing nanomaterials and to work with toxicologists to design new assays[11].

For the field to progress, it is necessary for all papers to report detailed characterization of the materials used so that data from the toxicity studies can be properly interpreted, reproduced and compared by others[12]. And the big challenges in the coming years are to understand how physical and chemical properties of nanomaterials govern their interactions and responses, and to inform the public on the benefits and risks associated with the use of nanomaterials. ❐

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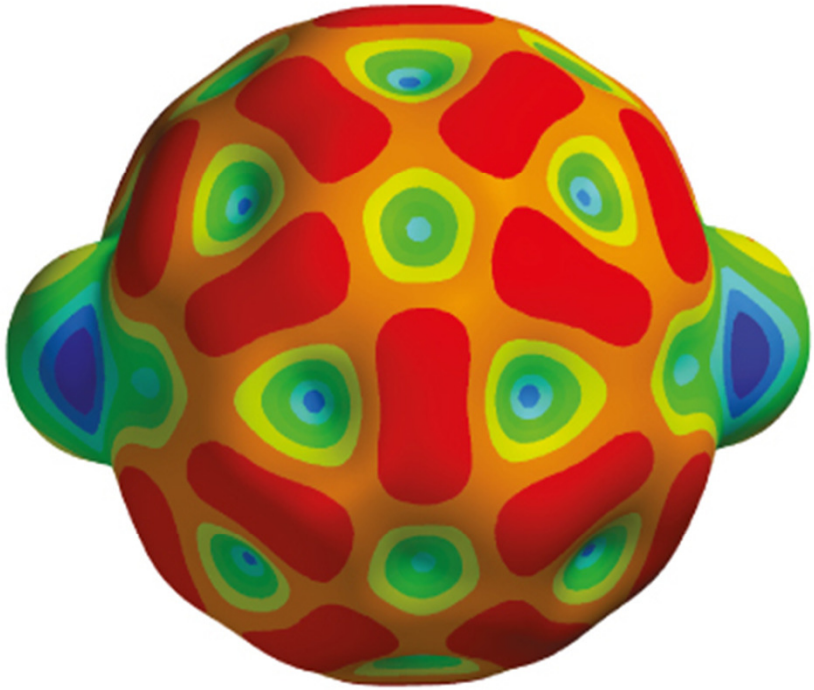

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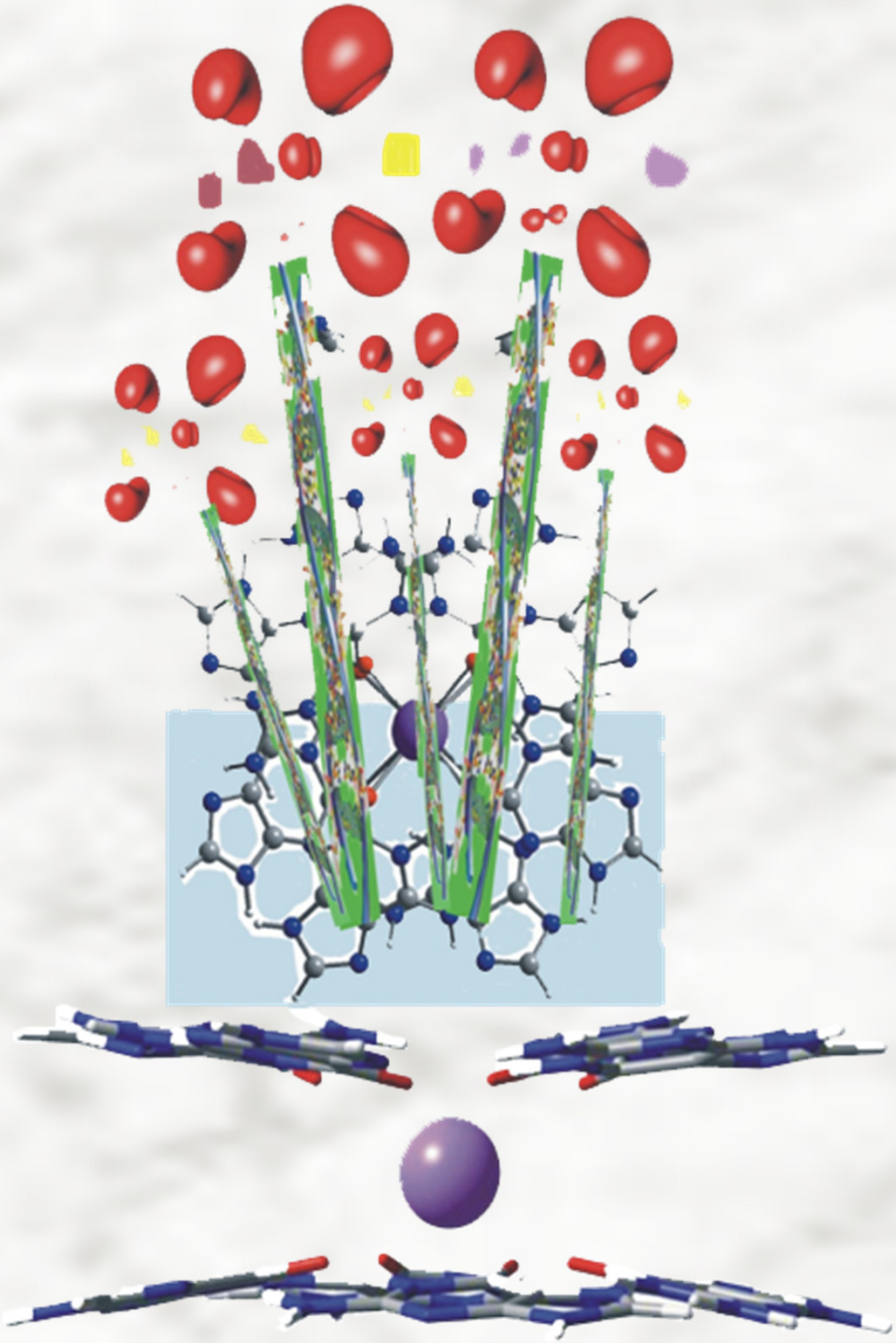

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