The Art of Nanotoxicology

Nanoscience and Nanotechnology

by

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Introduction

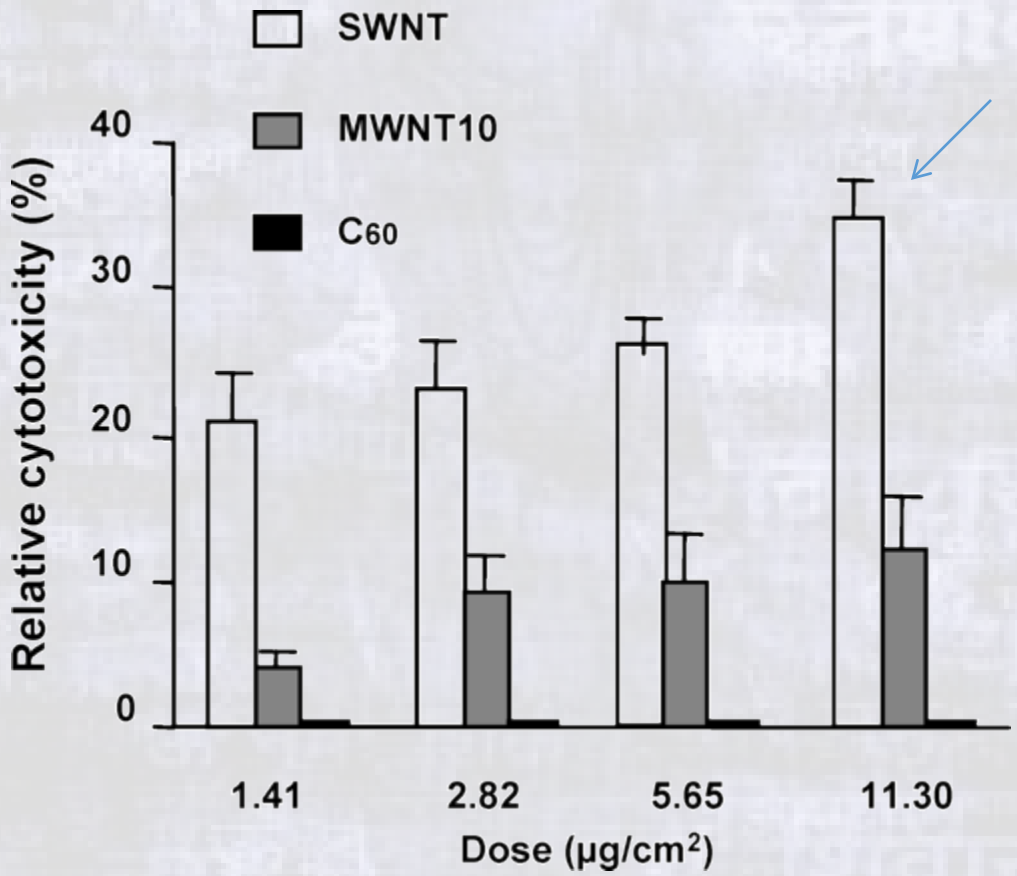
- medicinal, pharmaceutical applications --- biosensing, MRI, optical detection, drug delivery systems, etc.,
- nano market will grow exponentially($2.6 trillion in 2014)
- 3 -4 nanotechnology computer projects are introduced into the market.

- Toxicology (from the Greek words *toxicos and logos) is the study of the adverse effects of chemicals on living organisms. It is the study of symptoms, mechanisms, treatments and detection of poisoning, especially the poisoning of people and the environment pollution*

toxic ---degree to which a substance can damage an organism.

- cytotoxic - quality of being toxic to cells.
- neurotoxic - to cause damage to nervous tissue
- genotoxic - Any substance capable of causing damage to cellular DNA and thus producing mutations or cancer.
- ecotoxic - how chemicals affect the environment and the organisms living in it.
- examples -- SWCNTs, MWCNTs , fullerenes suspended in water will produce cytotoxicity to human cell line, SiO_2 TiO_2 , ZnO_2 pulumonary inflammation to humans, silver nanoparticles might affect microorganisms in environment.

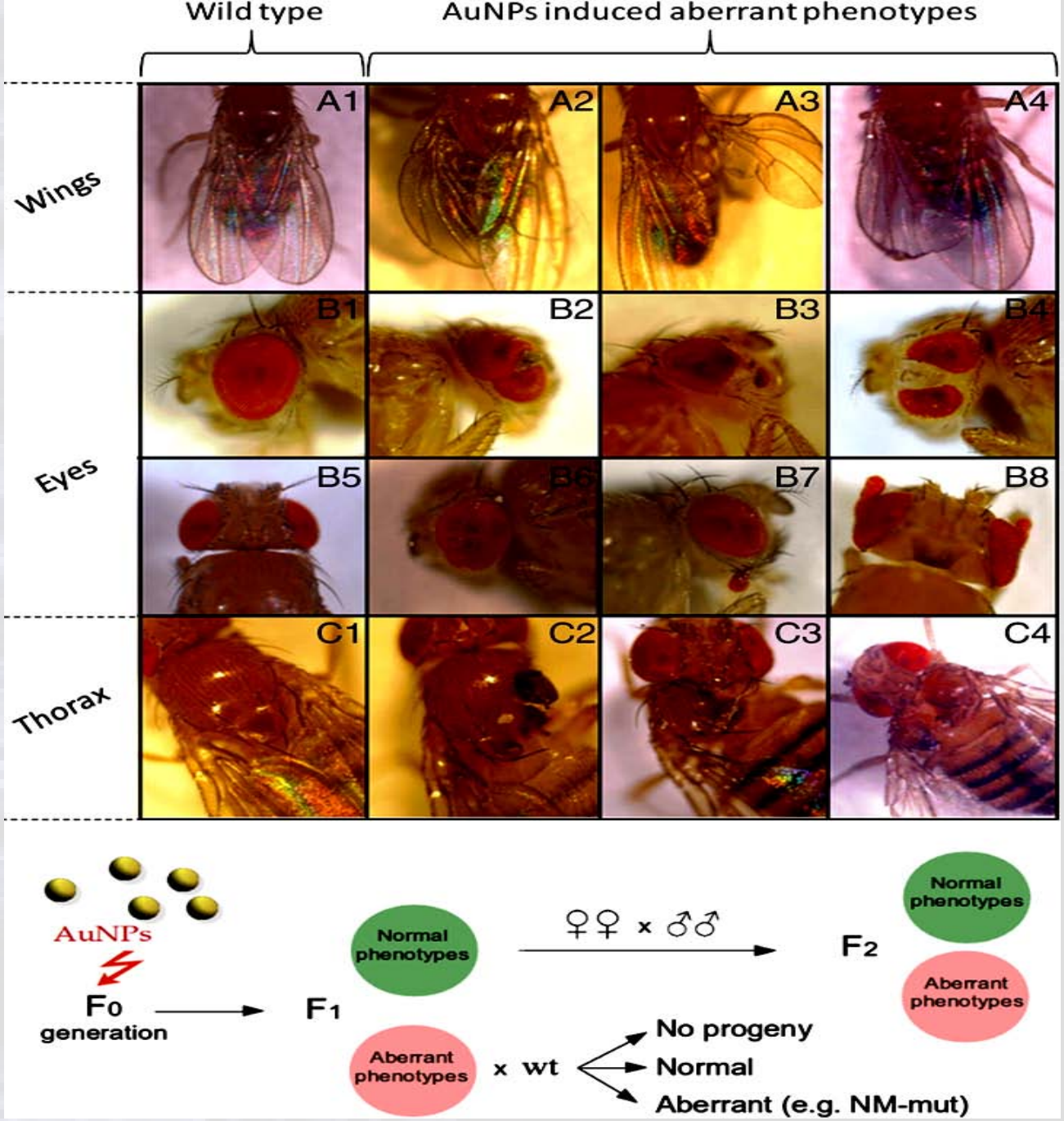
Cytotoxicity of carbon NP's in SWCNT, MWCNT, and Fullerene



The cytotoxicity increases by as high as 35% when the dosage of SWNTs was increased by 11.30 μg/cm^2. No significant toxicity was observed for C_{60} up to a dose of 226.00 μg/cm^2. The cytotoxicity apparently follows a sequence order on a mass basis: SWNTs > MWNT10 > quartz > $C_{60(}$diameter =10-20 nm) alveolar macrophage(6 hr in vitro)

Genotoxic – *[Eg : use of gold nanoparticles(in-vivo study) I nduce aberrant phenotypes in Drosophila melanogaster*]

Reference :
Mutagenic effects of gold nanoparticles induce aberrant phenotypes in Drosophila melanogaster
Author : Giuseppe Vecchio.



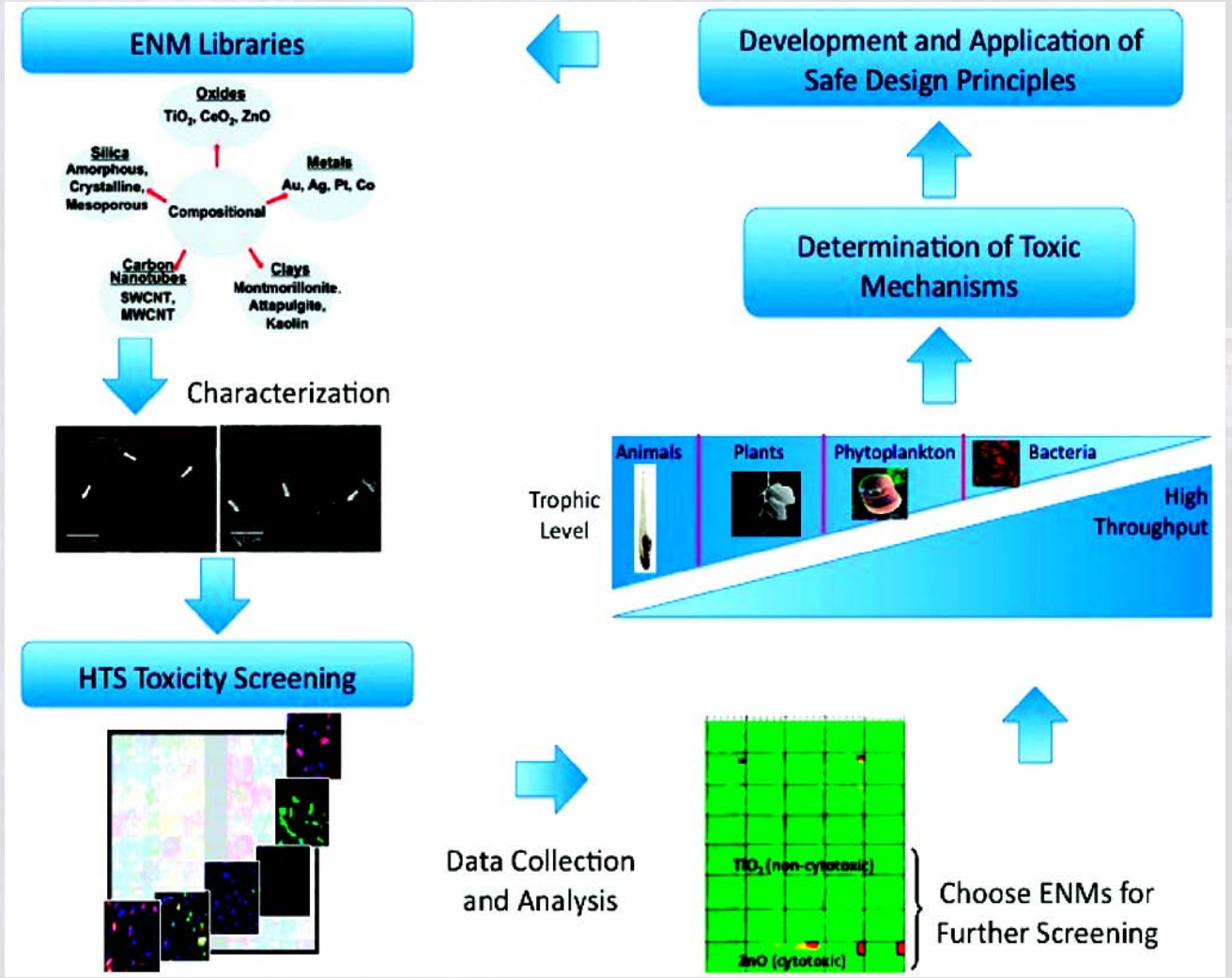
Ecotoxicology(example is impact of metal NP's on marine Phytoplankton)

- effects of two types of metal oxide nanoparticles, TiO_2 and ZnO, on population growth rates of four species of marine phytoplankton representing three major coastal groups (diatoms, chlorophytes, and prymnesiophytes). Titania NPs showed no measurable effect on growth rates of any species, while ZnO NPs significantly depressed growth rate of all four species. Our results suggest that effects of metal oxide NPs on marine organisms is likely to vary with particle type and organism taxonomy
- Ref : **Impacts of Metal Oxide Nanoparticles on Marine Phytoplankton** Robert J. Miller *, Hunter S. Lenihan , Erik B. Muller, Nancy Tseng , Shannon K. Hanna , and Arturo A. Keller

Commonly accepted and followed risk assessment procedures for ordinary chemicals include and disadvantage of nanomaterials use

- Exposure assessment - exposure assessment defines the sources, pathways, routes, and the uncertainties in the assessment.
- bad luck -- we do not have this date on nanomaterial, because in nanomaterial property if a function of shape, size, surface property and surrounding also
- lack of data on time exposure to engineered nanomaterial
- Hazard assessment - requires a combination of hazard characterization and hazard identification information for the studied agents.
- Lack of sufficient information about dose- response relationship. The information is available on animal, where they project the results to human. One more thing is, here aniamls are exposed to nanoparticle, intake by inhalation, for short duration
- The ability of Np's to reach body through different administration
- Present information is for invitro, for short duration.. Not for long duration

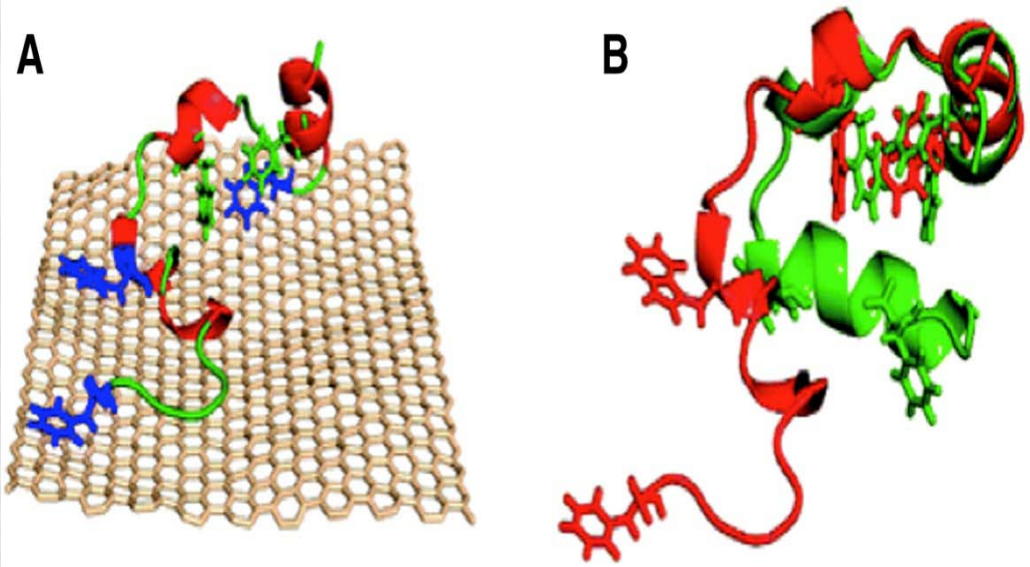
Strategy for determining the toxicity of various nanomaterials



Published in: Courtney R. Thomas; Saji George; Allison M. Horst; Zhaoxia Ji; Robert J. Miller; Jose R. Peralta-Videa; Tian Xia; Suman Pokhrel; Lutz Mädler; Jorge L. Gardea-Torresdey; Patricia A. Holden; Arturo A. Keller; Hunter S. Lenihan; Andre E. Nel; Jeffrey I. Zink; *ACS Nano* **2011,** 5, 13-20.
DOI: 10.1021/nn1034857

- Invitro - animal
- Invivo animal and human cell line
- World and european organisations like OESD, REACH,EPSA, SCCP, EPA have taken necessary steps to to describe necessary endpoints that contain information to assess nanomaterial risk assessment

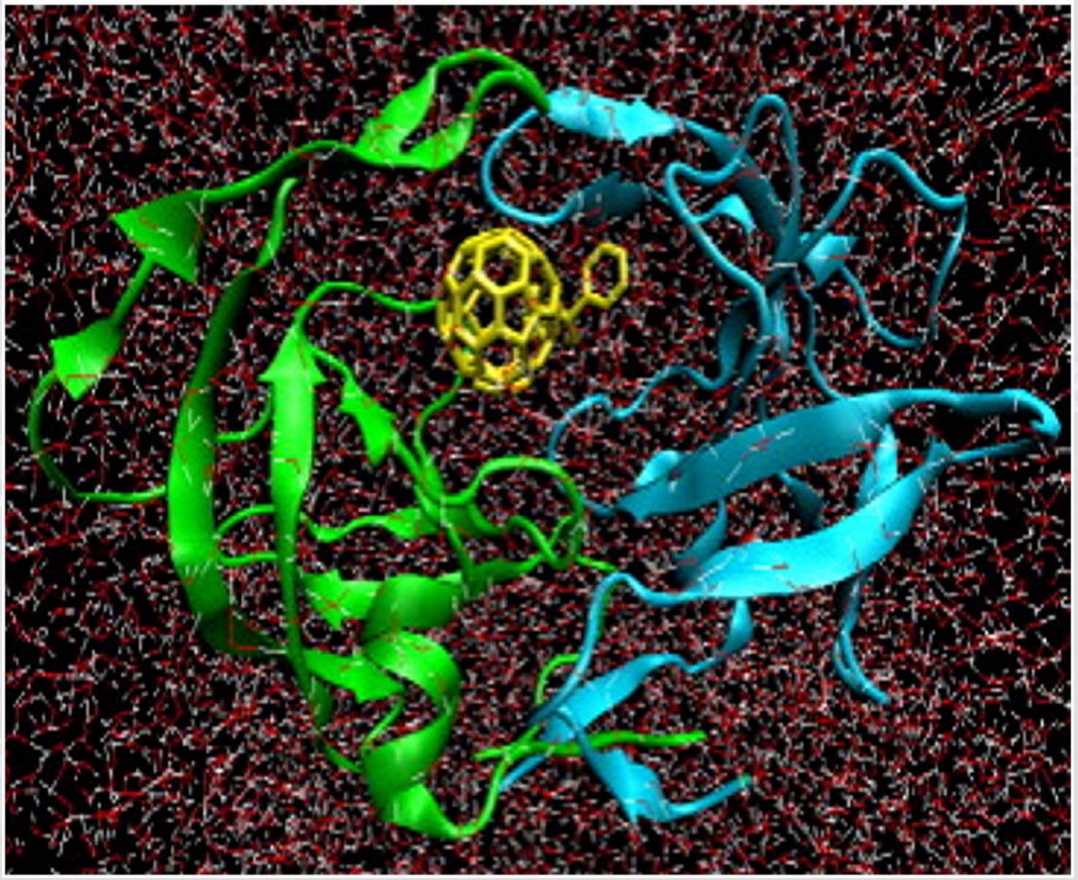
How modeling and simulations help you ?



(A) A typical structure of HP35 adsorbed on the graphene surface. Here, HP35 is shown as a cartoon with red helix and green loop, the graphene is shown as the cyan lines.
(B) The superposition of the adsorbed HP35 structure on graphene (red) with its native structure (green). The aromatic residues that form the π–π stacking interactions are shown in blue.

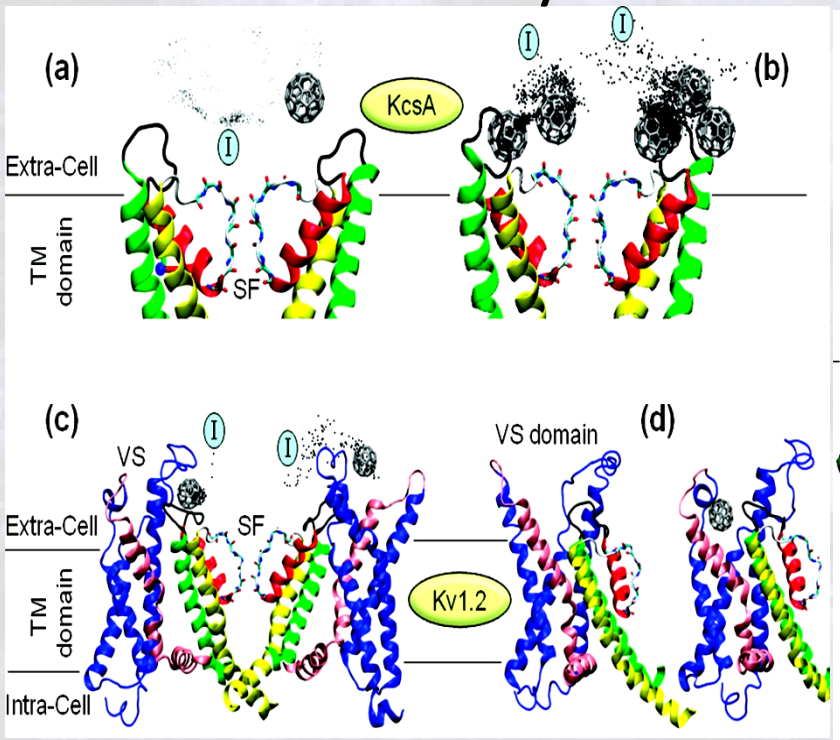
G. Zuo, X. Zhou, Q. Huang, H. Fang, R. Zhou **Adsorption of villin headpiece onto graphene, carbon nanotube, and C60: effect of contacting surface curvatures on binding affinity** J. Phys. Chem. C, 115 (2011), pp. 23323–23328

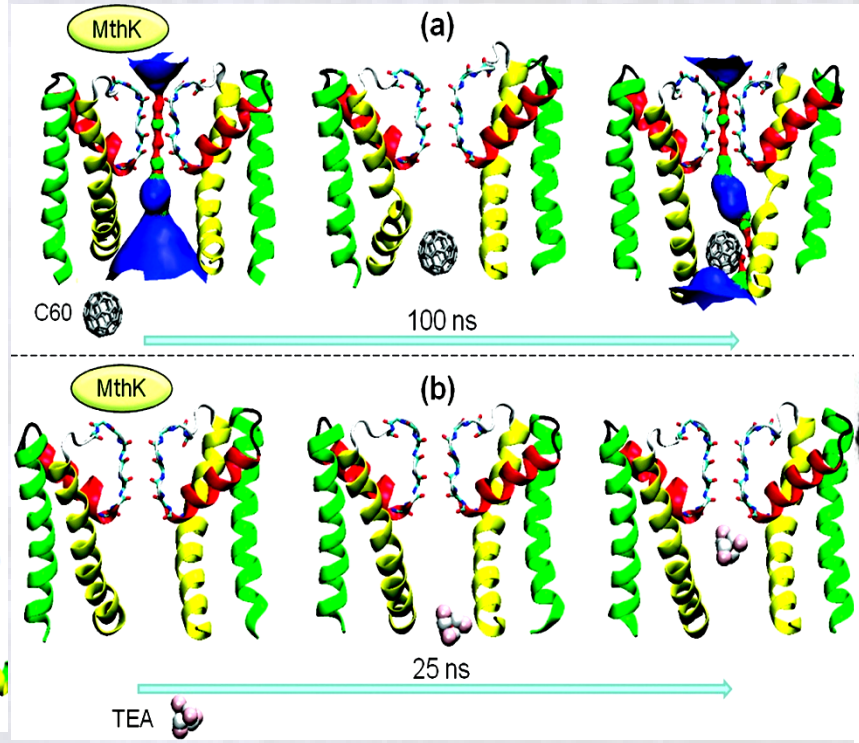
Fullerene derivatives promising inhibitors for HIV-1 PR(aspartic protease), which in turn is an important target enzyme for AIDS Drug design

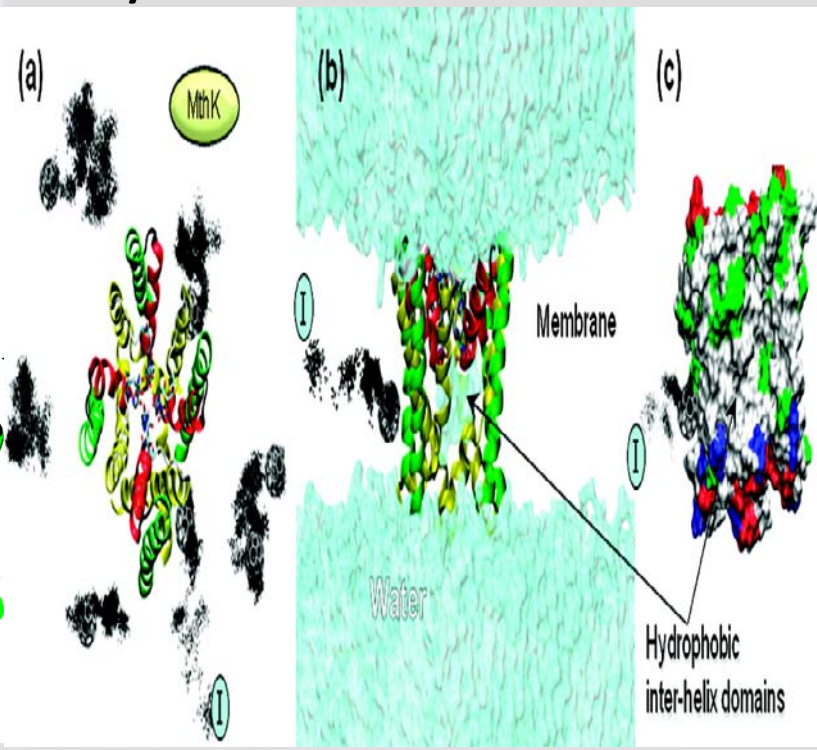


S. Durdagi, T. Mavromoustakos, M.G. Papadopulos **3D QSAR CoMFA/CoMSIA, molecular docking and molecular dynamics studies of fullerene-based HIV-1 PR inhibitors**

Fullerenes may act as blockers or modulators of a variety of K^+channels



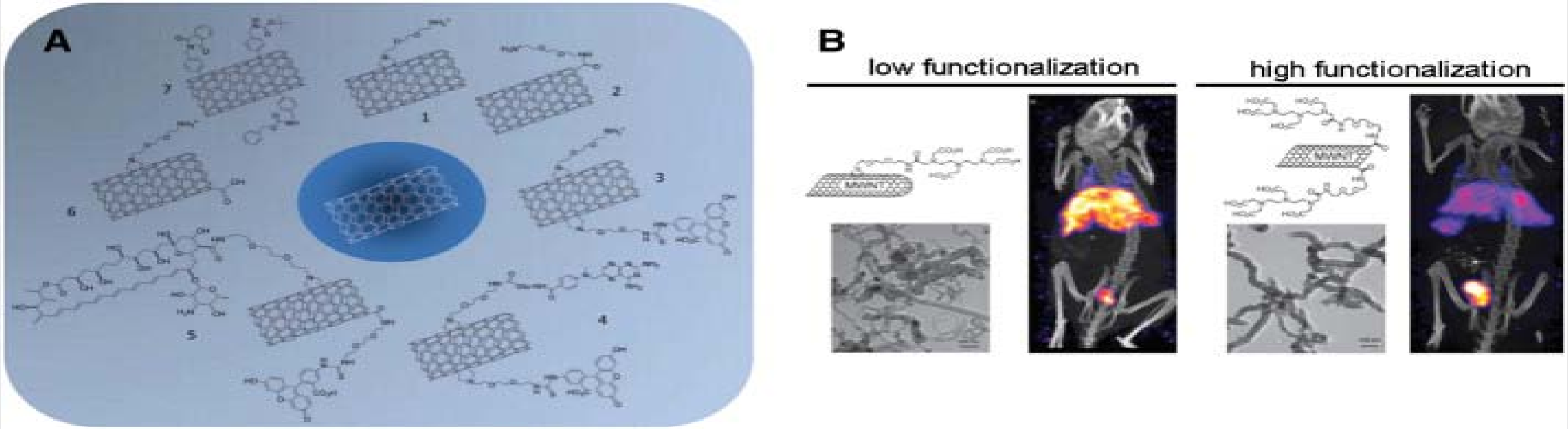




Binding of C60 to the extracellular domains of KcsA (top) and Kv1.2 (bottom). The dots represent the position of C60 along the MD trajectory sampled every 0.1 ns starting at position I and ending at the displayed C60. Binding of (a) monomeric and (b) aggregates of C60 to the extracellular part of KcsA. (c) Binding of C60 to the voltage sensor domain (VSD) of Kv1.2. (d) Structure of the Kv1.2 VSD (d,left) before and (d,right) after binding of C60. For clarity, the membrane lipids and the water molecules are not shown and only two monomers of each channel are displayed. KcsA channel is shown truncated in order to focus only on its extracellular side.

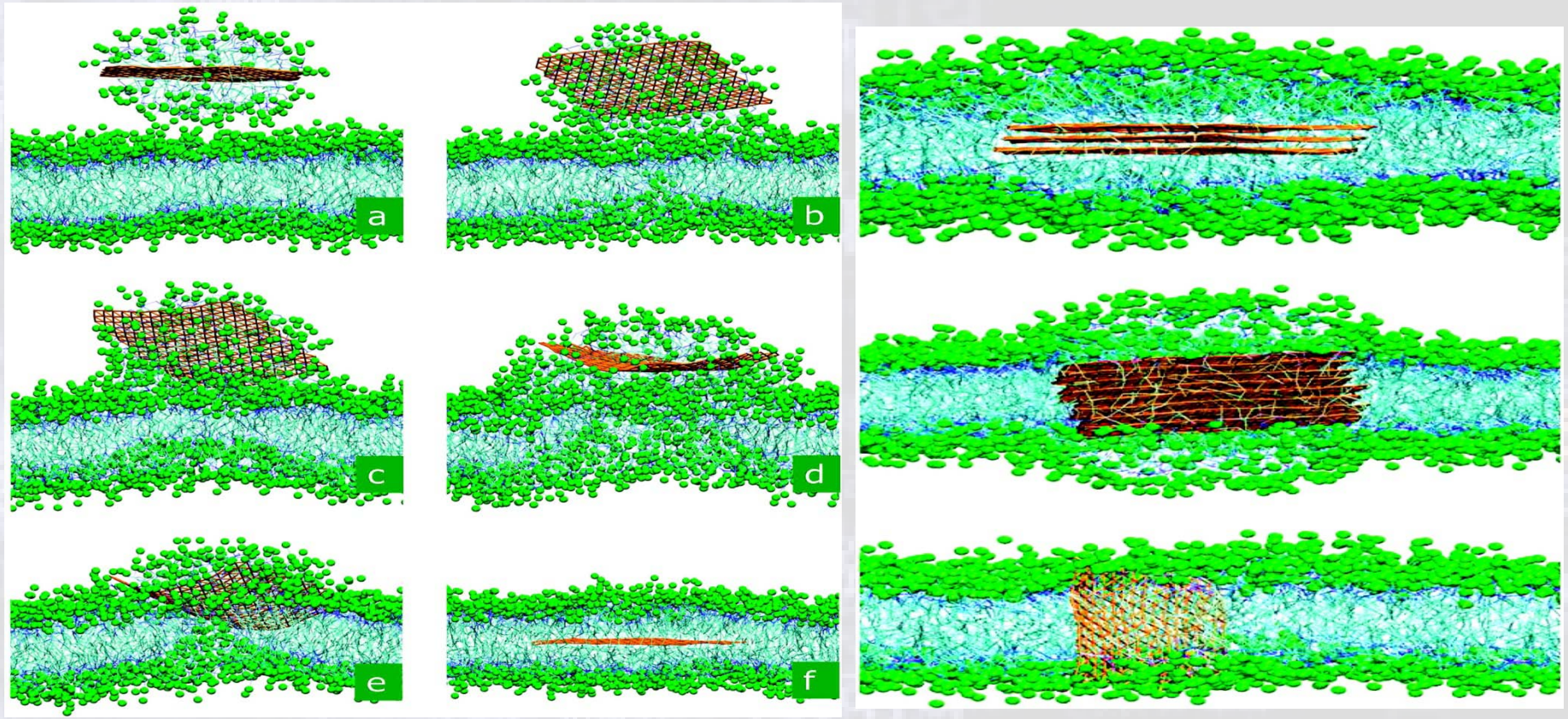
Published in: Sebastian Kraszewski; Mounir Tarek; Werner Treptow; Christophe Ramseyer; *ACS Nano* **2010,** 4, 4158-4164.
DOI: 10.1021/nn100723r

How toxic substances can be modified to make non-toxic?



Structures of various important carbon nanomaterials: fullerene (C 60), single-walled carbon nanotube (SWCNT), multi-walled carbon nanotube (MWCNT), carbon nanohorn, graphene, few layer graphene and graphene oxide chemical modifications on carbon nanotubes. Structures 1 and 2 correspond to mono-functionalized CNTs; structures 3–6 correspond to bi-functionalized CNTs; and structure 7 corresponds to tri-functionalized CNTs Functionalized CNT biodistribution in mice (with low and high degree of functionalization). Chemical structure, TEM images of the f-MWCNTs and SPECT/CT images of live animals injected with radiolabelled f-CNTs indicate that high liver accumulation (left) can be modulated leading to increase in urinary excretion (high bladder signal) as degree of functionalization increases (right)

Agnieszka Gajewicz , Bakhtiyor Rasulev , Tandabany C. Dinadayalane , Piotr Urbaszek , Tomasz Puzyn , Danuta Leszcz...
Advancing risk assessment of engineered nanomaterials: Application of computational approaches



Self-insertion of graphene monolayer inside the phospholipid membrane. In the displayed process, a graphene micelle merges with the membrane and releases the monolayer, which penetrates the membrane. The snapshots are taken at *t*a−f = 2.9, 52.4, 120.0, 299.2, 356.4, and 516.4 ns, respectively. (Top to bottom) Equilibrated composite structures with three and eight graphene monolayers (≈5.9 × 6.2 nm2), as well as a vertically oriented graphene sheet (≈4.3 × 5.9 nm2), with two-edge functionalizations by the polar heads of POPC lipids, all stabilized inside the POPC bilayer membrane.

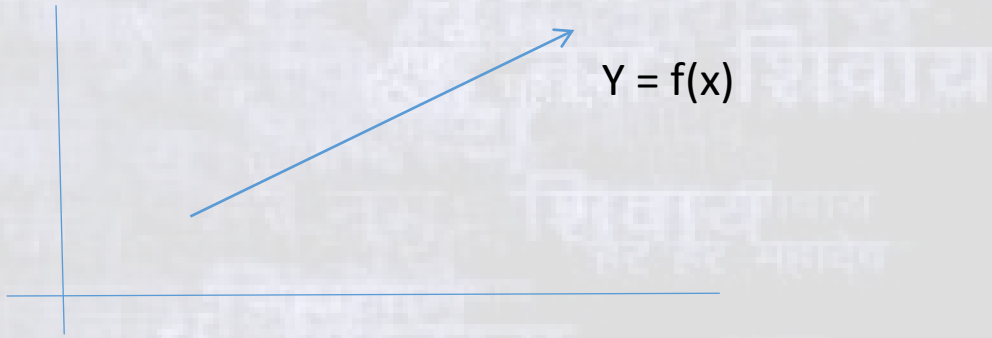
Published in: Alexey V. Titov; Petr Král; Ryan Pearson; *ACS Nano* **2010,** 4, 229-234.
DOI: 10.1021/nn9015778

Quantitative Structure-Activity Relationships for nanomaterials

- insufficient toxicity information makes difficult to develop the structure–toxicity relationship of nanoparticles.
- REACH legislation introduced in Europe allows computational tools in replacing experimental tests in some cases. Some computational tools for example, QSAR. are essential for increasing throughput, reducing the burden of animal testing, providing details of the toxicitymechanisms, and generating novel hypotheses for risk assessment.

How qsar model is developed(in general)

- y = f(x) , where y is defined as Molecular structure. And f(x) is defined as a function of Molecular discriptors, or variables.. We need to use statistical analysis to above equation to predict new structure behaviour.



Insilico tools for toxicity prediction- toxicity data can be obtained from databases

Database	illustrative example
Single literature database	Skin sensitization data from a single source Of a guinea pig
Literature compilations	Historical databse for lymph node assay – CPDB
Freely accessible compilations of toxicity Information	ToxNet, AMBIT
Refined (and hence potentially higher quality) Databases	DSSTox, European Union Framework Projects, e.g., the CAESAR project
Databases developed for regulatory purpose	European chemical Substances Information System()ESIS
Commercial databases	Leadscope (including FDA) databases VITIC database from Lhasa Ltd.

Free	COMMERCIAL
TEST, DRAGON, VEGA, OCHEM , CDK	• *CODESSA,* • *DRAGON,* • *GRIN/GRID* • *HYBOT-PLUS,* MOLCONN-Z • *POLLY,* •TSAR, ADAPT
CLASSIFICATION DISCRIMINANT ANALYSIS CART KNN FUZZY LOGIC BAYESIAN SELF ORGANISSING MAPS SUPPOR VECTOR MACHINES	*REGRESSION* *MULTIVARIATE ANALYSIS* *PARTIAL LEAST SQUARES* *NEURAL NETWORKS* *OTHERS(PRINCIPLE COMPONENT ANALYSIS, GENETIC ALGORITHMS)*

SOFTWARES USED TO CALCULATE DESCRIPTORS

the models that are used are, CLASSIFIERS AND REGRESSION TECHNIQUES USED TECHNIQUES IN QSAR:

EndPoint	Software/ Methods
Carcinogenecity	SARs(OncoLogic)
Skin absorption	QSARs(linear regression models)
Environmental/ Ecological effects	EPISUITE and PBT profiler
Physiochemical Properties	KOWWIN
Acute and Chronic Toxicity	ECOSAR
Endocrine disruption knowledge base	EDKB

AGENCY	TESTS
ATSDR (AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY)	Toxicity prediction - QSARs based on PBPK • *Benchmark Dose (BMD) for human health effects*
FDA (*Food and Drug Administration - Dept. of Health & Human Services*)	Carcinogenicity - data from regulatory submissions used to develop MULTICASE
NTP (*National Toxicology Program)*	Carcinogenicity - tested commercial software

When it comes to europe---

the project REACH legalisation consultancy in spain is

REACH Monitor, Spain

Experimental properties for possible use as descriptors in nano-QSARstudies

• 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 pore size diameter	Particle filtration

Difficulties faced by nano-QSAR

- The first one is lack of sufficiently numerous and systematic experimental data, while the second one is very limited knowledge on mechanismsof toxic action.

Outline of Nano-QSAR.

- If one considers the amount of available experimental data to be represented as a matrix where the rows are filled with specific nanoparticles (ZnO, SiO,SWCNT, C60, etc.) and the columns with particular data (EC50, Zetapotential, solubility, etc.), then, certain properties of nanomaterialsunder specific conditions and size represent another matter.
- the data for5 nm and 100 nm nano-TiO2 clusters should be treated like the resultsobtained for different species.
- Use of commercial softwares to generate structural descriptors
- Now use of statistical methods, algorithms to generate functinal property, which helps to predict new nanomaterial to be toxic or not.

The CORAL software: principles, results, perspectives

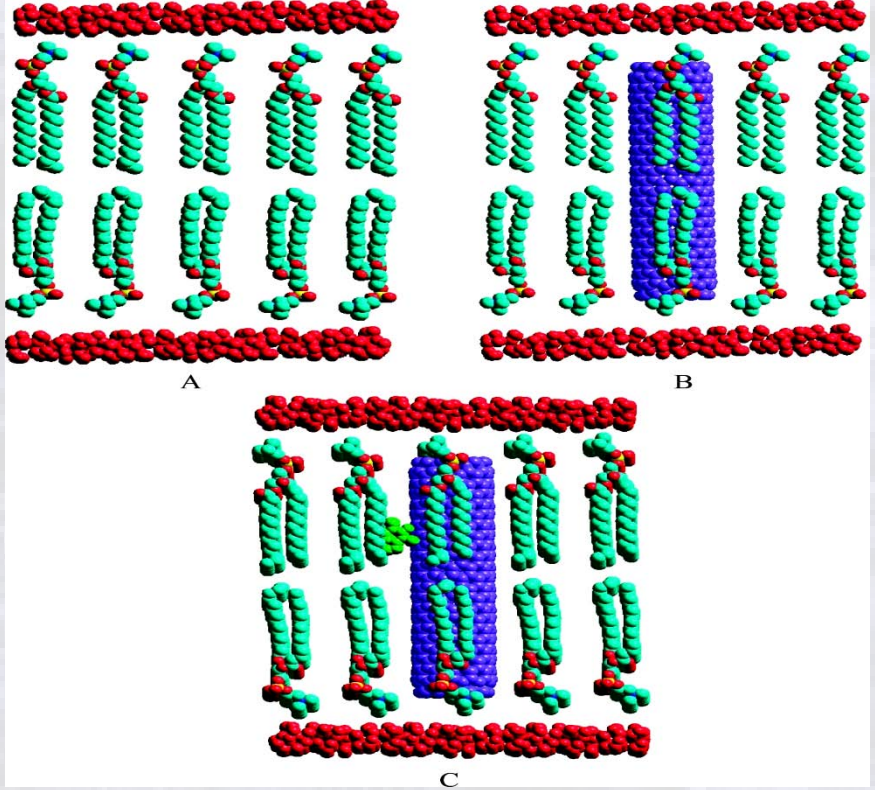
- The CORAL software has been used as a tool for building up the quantitative – structure property / activity relationships (*QSPR/QSAR) of a number of various endpoints: inhibitors of human serine proteinases , anticonvulsant agents , anti-malaria activity , anticancer agents , toxicity toward Daphnia magna , mutagenicity , toxicity toward rat , bioconcentration factor , carcinogenicity , solubility and anti-HIV-1 activity of fullerenes . Our aim is to briefly inform about the CORAL software, in "the first approximation*

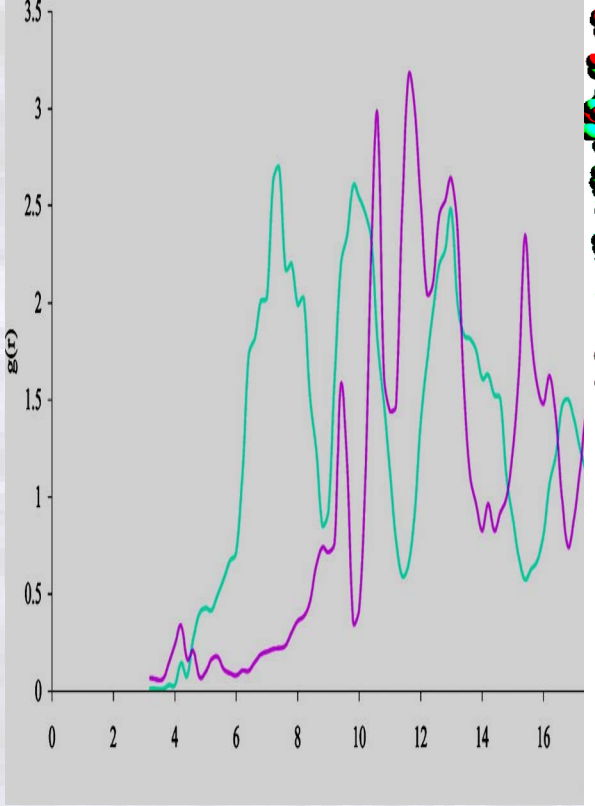
Genetic Algorithms in QSAR for REACH

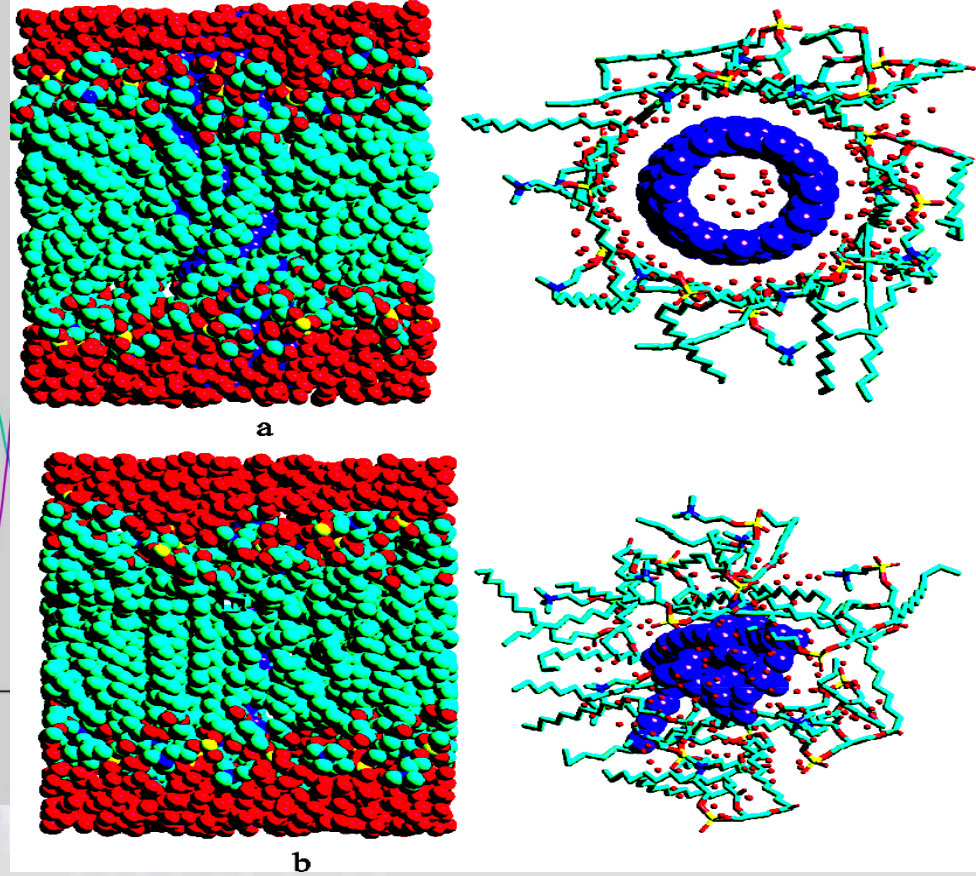
- **Random creation of the initial population**
- **Fitness-based reproduction**
- **Termination**

Example to explain how Computational QSAR can give valuable information to nanotoxicity.

- **Identification of Possible Sources of Nanotoxicity from Carbon Nanotubes Inserted into Membrane Bilayers Using Membrane Interaction Quantitative Structure –Activity Relationship Analysis**







Schematic representations of the different bilayer assemblies studied with hydrogens deleted. (A) DMPC, bilayer assembly; (B) DMPC, nanotube assembly, with the nanotube colored as violet; (C) DMPC, nanotube penetrant, with nanotube colored as violet and the penetrant (caffeine) colored as green. CDFs of the DMPC bilayer assembly with the nanotube inserted (violet) and without the nanotube (cyan). a) Side view of the nanotube in the DMPC bilayer with waters on the top and bottom and all hydrogen atoms suppressed in the rendering. The nanotube is shown in violet. Top view of the nanotube in the DMPC bilayer with only the first layer of DMPC molecules around the nanotube shown. There is an obvious cylindrical hole formed around the nanotube. (b) Side view of center DMPC of the DMPC bilayer with waters on the top and bottom. All hydrogen atoms have been suppressed. The center DMPC is shown in violet. Top view of the center DMPC of the DMPC bilayer with only the first adjacent DMPC packing layer shown. There is no cylindrical hole around the center DMPC molecule.

Published in: Jianzhong Liu; Anton J. Hopfinger; *Chem. Res. Toxicol.* **2008,** 21, 459-466.DOI: 10.1021/tx700392b

Conclusion

- The problem with Application of QSAR in the field of Nanotechnology is, protocols were not generalised, they vary with experiement to experiment. We get lot of variables.
- There were few research groups who tired to evaluate the the descriptors, they introduced lot of variables. They could predict lot of models, stating that they found a relation to describe the toxicity.
- Guidelines has to be clear, interaction between theoritical and experiemental scientists is must , to find a solution to reduce toxicity from materials.

THANK YOU .