Insights on the interactions of nanoparticles with cellular membranes

Paolo Elvati, Angela Violi
Department of Mechanical Engineering
University of Michigan, Ann Arbor, MI 48109 USA

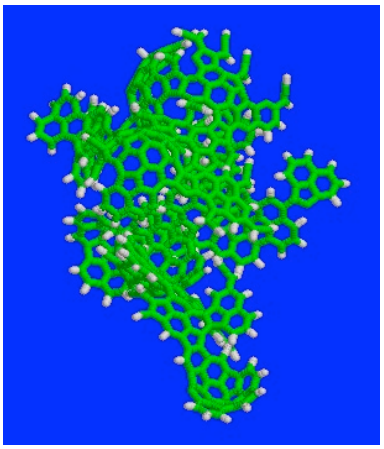
Nanoparticles are of a scale and chemical composition to be "at home" in many biomolecular environments. They can have hydrophobic, hydrophilic, or amphiphilic character, much like important biological molecules such as lipids and proteins. Moreover, they are often of a shape and size that is not unlike that of proteins and other key biomolecules. Only recently have critical questions regarding the potential human health and environmental impact of nanoparticles been raised.

Without question, the process of combustion is the dominant pathway through which mankind continuously injects particulate matter into the atmosphere at the present time. These combustion-generated particles are present not only in a very large amount, but are produced, at the smallest scale, in the form of clusters with nanometric dimensions. Modern diesel engines are a major source of these combustion-generated nanoparticles. Although the total mass of particulate emissions has been significantly reduced with improvement of combustion efficiency and emissions control systems, the very small nanoparticles are exceedingly difficult to control by the emission systems typically installed on vehicles. In addition, the current emissions regulations are mass-based and do not address the presence of nanoparticles. In fact, studies conducted on exhaust measurements of particle size and number concentration data from diesel engines,[1-5] show that nanoparticles represent only 0.1 to 1.5% of particle volume (mass) but 35 to 97% of the particle number.[6] Environmental regulations of diesel engines and other combustion sources based on particle mass or volume instead of particle number will nearly *entirely miss* the dangerous nanoparticle component of such emissions.

Given that such great quantities of carbonaceous nanoparticles are being introduced into the atmosphere by combustion source, the question naturally arises as to their environmental fate and interactions with living systems of humans. The high number concentration and small size of the carbonaceous nanoparticles will clearly lead to high rates of deposition deep in the lung.[7] Recent studies on the mechanisms by which ultrafine particles act on biological systems [8] reveal that the particle deposition on the epithelial cells in the lungs trigger a number of responses: cell activation leading to inflammation; production of cytokines (proteins) that stimulate the release of fibrinogens, which bind to platelets, contribute to their aggregation, and enhance their ability to clot; and stimulation of nerve cells that leads to changes in the nervous system's control of breathing and heart rate. Therefore, nanoparticles emitted by diesel engines and other combustion sources are a health concern because of both their size and the carcinogens with which they are associated.

The goal of this work is therefore to explore the interactions of carbon-based nanoparticles with biomolecular structures representative of those at the cellular scale, using atomistic simulations.

The formation of carbonaceous nanoparticles from diesel is the result of the accumulation of particle mass via chemical reactions with gaseous precursors obtained through the combustion of the fuel. This process occurs simultaneously with the growth of particle size by collision among polycyclic aromatic hydrocarbons molecular species and clusters. Recently our group has developed a new approach to study the transition from gas-phase to nanoparticles, involving the coupling of Kinetic Monte Carlo (KMC) and Molecular Dynamics (MD) methodologies.[9-11] The use of atomistic simulations allows us to follow the transformations that occur during nanoparticle formation in combustion environments in a chemically-specific way, providing information on both the final chemical structure and the configuration of the nanoparticles and young soot particles.



The figure at the right shows a typical nanoparticle obtained through the use of the AMPI code in a benzene-oxygen laminar premixed flame. The coupling between the KMC and MD modeling approaches provides a key to the multiple time-scales involved in the formation of nanoparticles, spanning pico- or nanoseconds for intramolecular processes that can occur for example on a particle surface to milliseconds for intermolecular reactions. The capability of the AMPI code has been validated in different combustion conditions.

Leveraging our ability to model the formation of combustion-generated nanoparticles, we investigated their interactions with cellular membranes. It has been calculated that the buckyball molecule possesses an exceptionally high passive permeability coefficient value through a lipid membrane, surpassing that of the aforementioned smaller molecules by an order of magnitude. In this context, we also consider the permeation of a C_{60}-sized, combustion-generated, pollutant-type particle, due to the enhanced probability of environmental exposure.

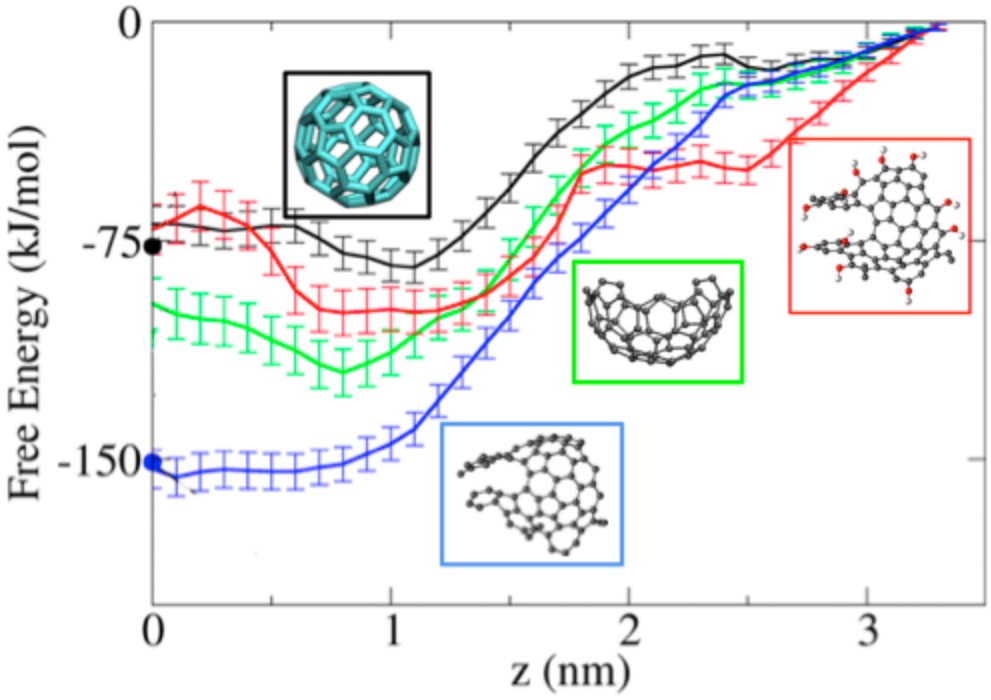
The free energy of interaction between nanoparticles and cellular membranes provides information on the ability of nanostructures to pass through membranes designed to act as barriers.[1213] This possibility can create many opportunities for toxic effects to occur.

Four nanoparticles were included in this study: buckminsterfullerene C_{60}, a representative combustion-generated NP termed NanoC ($C_{68}H_{29}$), the same particle with with OH (NanoOH), and an open C_{60} fullerene termed, Open-C_{60}.

For MD simulation, the DL_POLY 2.17 GUI was used to generate nanoparticle intramolecular forces. The representative membrane was comprised of 3:1, mixed phase, dimyristoylphosphatidylcholine (DMPC), cholesterol bilayer. The DMPC and cholesterol force fields were taken from the United Atom OPLS (UA-OPLS). Water molecules were specified by the TIP3P parameterization.

The initial bilayer system configuration consisted of a 3.26 x 4.37 x 7.27 nm^3 box containing 48 DMPC molecules interspersed with 16 cholesterol molecules, and solvated above and below with 1372 H_2O molecules. The bilayer norm was defined to be parallel with the *z*-coordinate axis, with distance z originating from the bilayer center and extending 3.4 nm into the aqueous phase.

The figure below reports the free energy profiles of 4 nanoparticles permeating a DMPC/cholesterol bilayer. As a hydrophobic molecule, C60, would be expected to possess greater stability in the alkyl tail group region of the bilayer, which is confirmed by inspection of the relative depth of the free energy potential.



The free energy profiles of combustion-generated particles, NanoC and NanoOH, differ in many respects from C_{60}, however, there are similarities at the membrane interface. The absence of a permeation barrier from the aqueous phase into the head group region and similar free energy slope, indicates comparable predicted timescale of particle uptake as C_{60}. In the bilayer alkane core, however, the calculated free energy minimum of NanoC differed in position from C_{60} and was found to possess significantly greater binding. Differences in the interaction energetics of C_{60} and NanoC may be attributable to differing sizes and/or morphologies of the two molecules.

The results indicate that the C_{60} may not adequately represent of a class of similarly sized, but morphologically differing combustion-generated nanoparticles with regard to assessments of nanotoxicity.

Acknowledgment
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Formation and Uptake of Environmental Nanoparticles

Angela Violi

Departments of Mechanical Engineering, Chemical Engineering and Biomedical Engineering

University of Michigan
Ann Arbor, MI USA
Research group: www.umich.edu/~avioli

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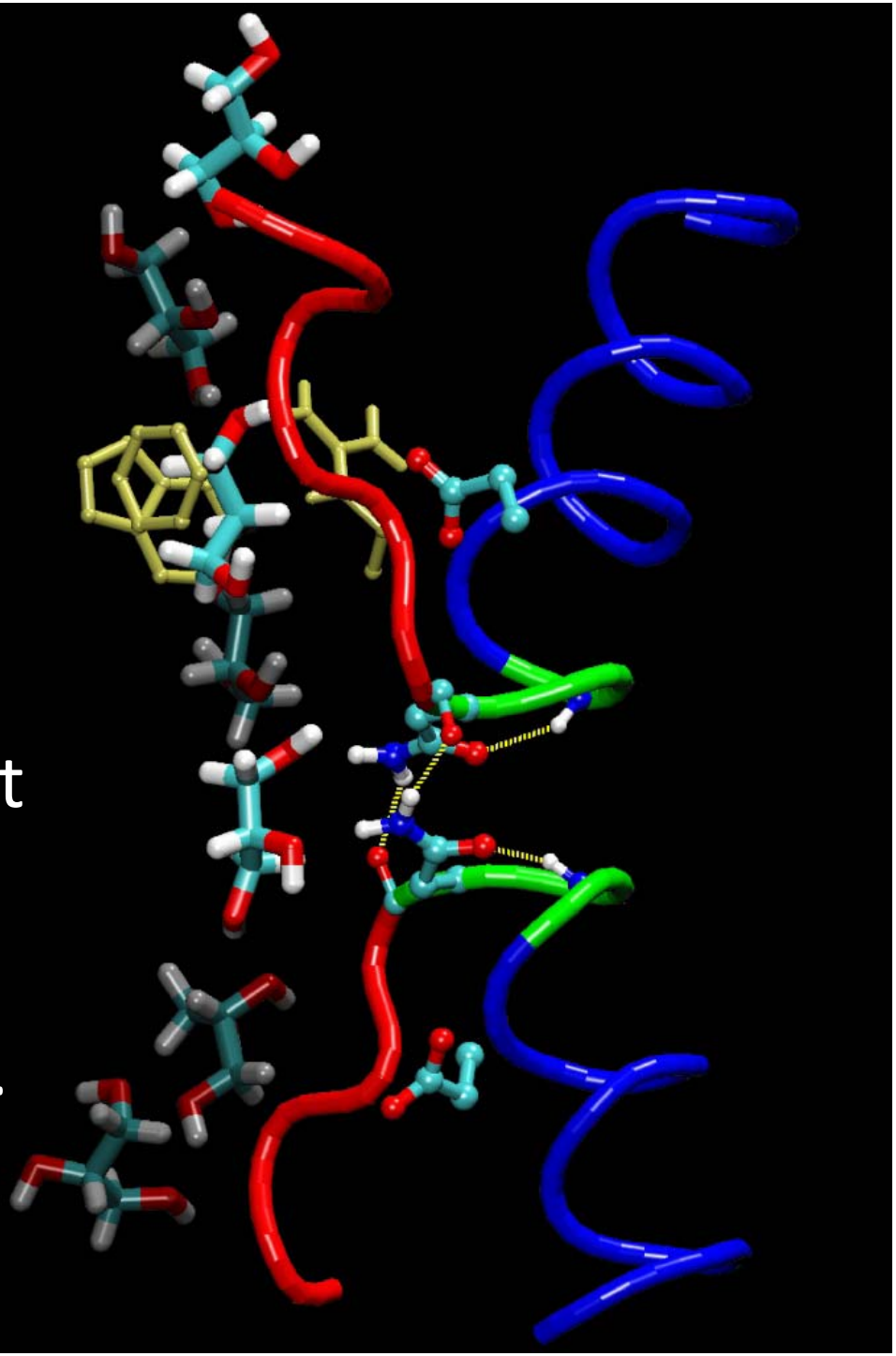
16th ETH Conference Combustion Generated Nanoparticles

Molecular Dynamics

It investigates the physical movement of atoms and molecules.

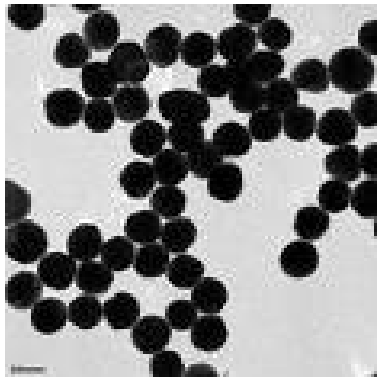
The time evolution of a set of interacting atoms is followed by integrating their equations of motion.



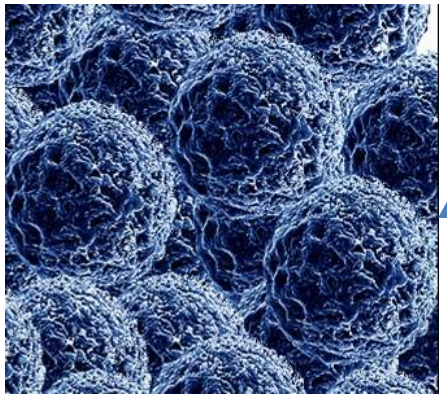
Objectives

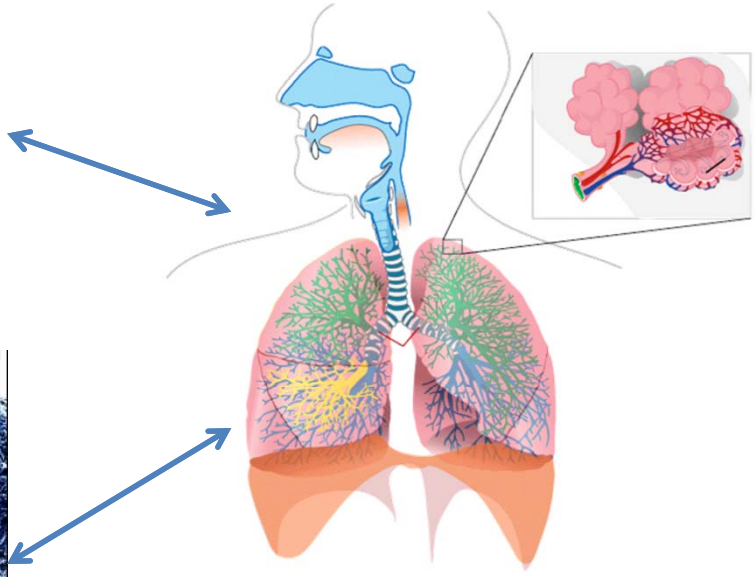
Investigate the molecular mechanisms of interactions between nanoparticles and biomolecular assemblies

Combustion-generated nanoparticles



Engineered nanoparticles





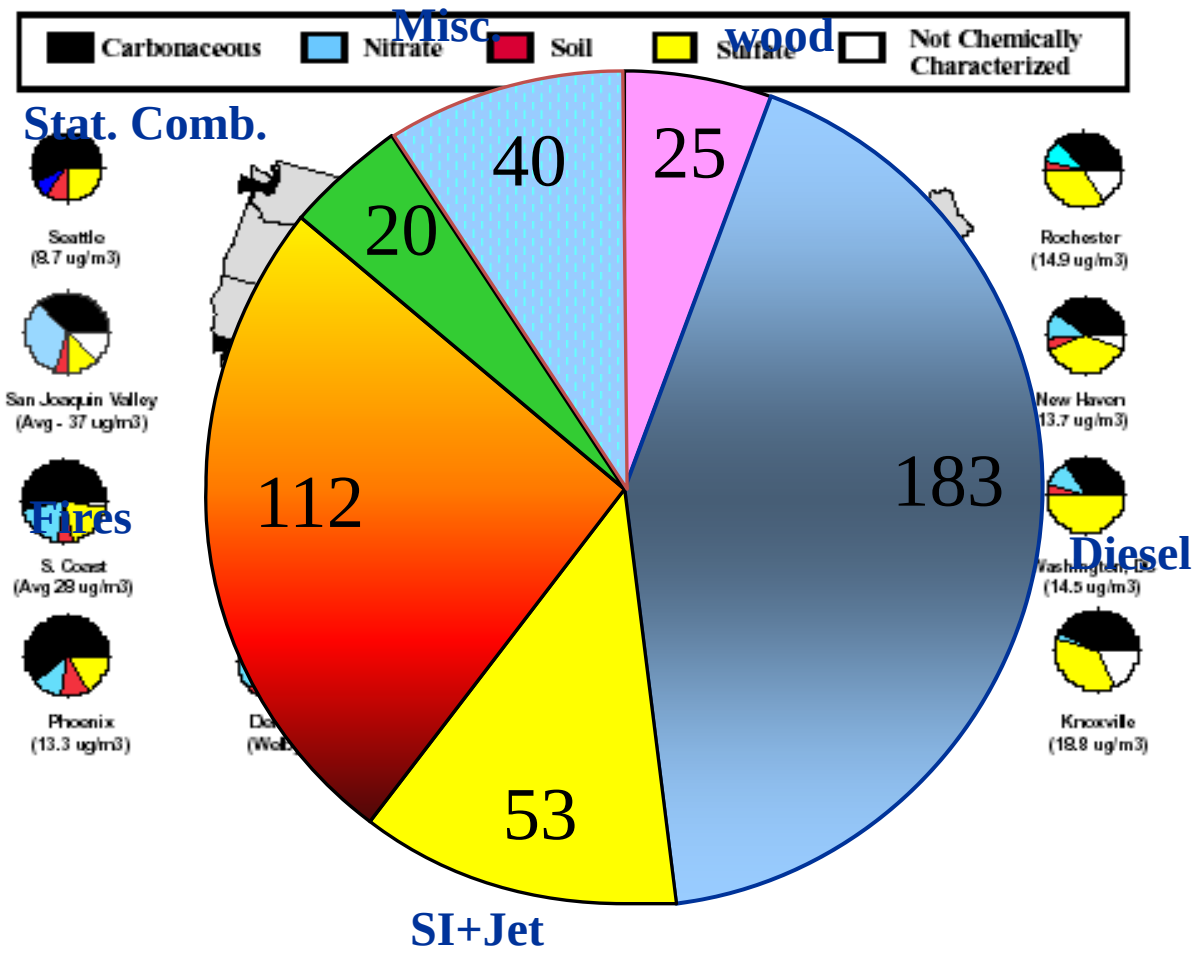
Risk Assessment

- RISK = HAZARD + EXPOSURE (ASSESSMENT)
- Too often, the ‘exposure’ part of this equation is omitted and hazard is equated with risk.
- Recent toxicity studies have demonstrated that high-dose, intratracheally-instilled, SWNT in the lungs of rats may produce unusual foreign-body tissue reactions.
- *But what is the exposure levels to carbon nanotubes?*
- *Particles are origin specific: chemistry and physics are different*

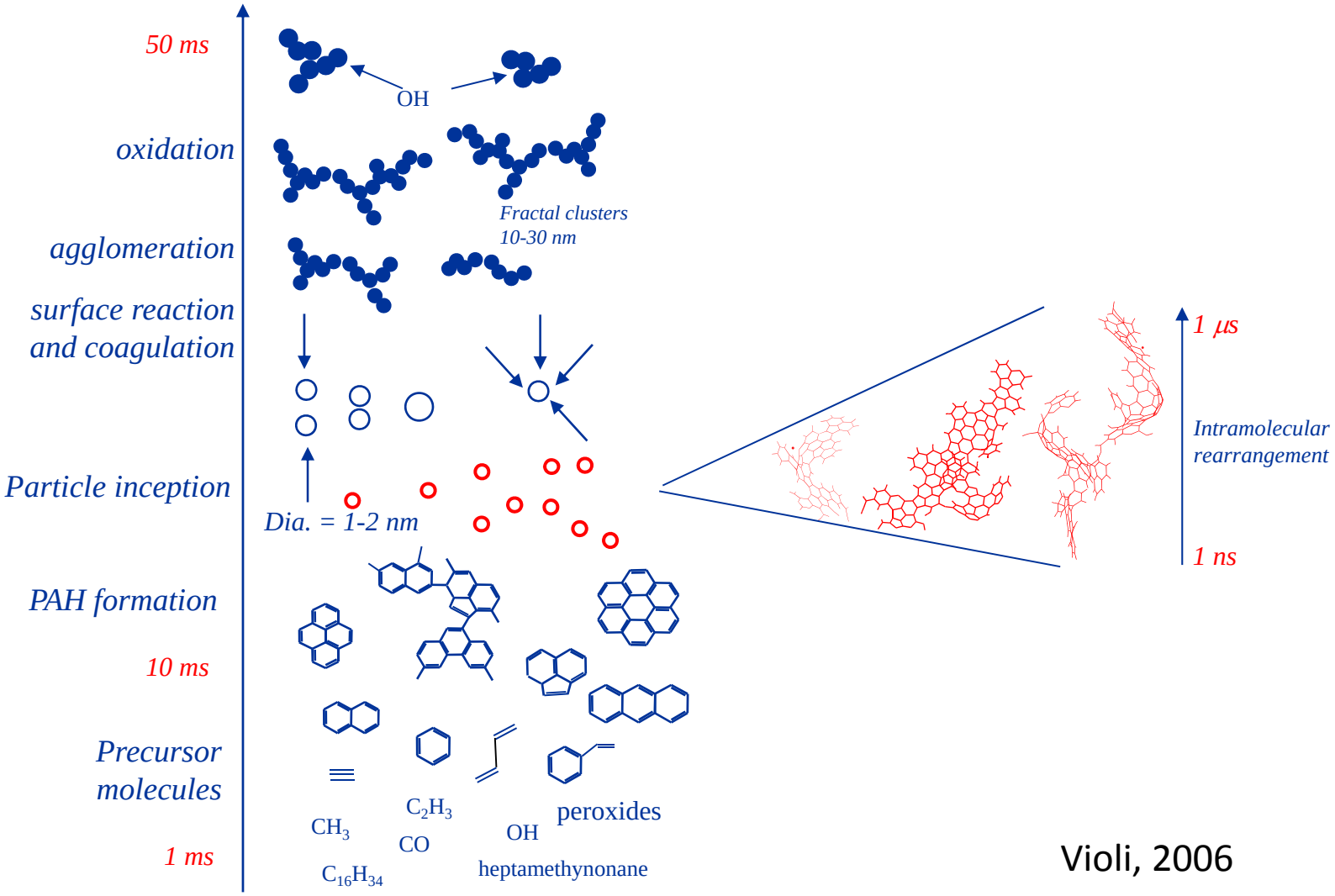
Main questions

- How environmental nanoparticles look like?
- What is the mode of **entry** into the membrane?
- Once inside, what kind of damage do they do?

Major classes of particulate matter: Carbonaceous particles dominate (Pace, EPA'05)



From few C to agglomerates of millions of C atoms



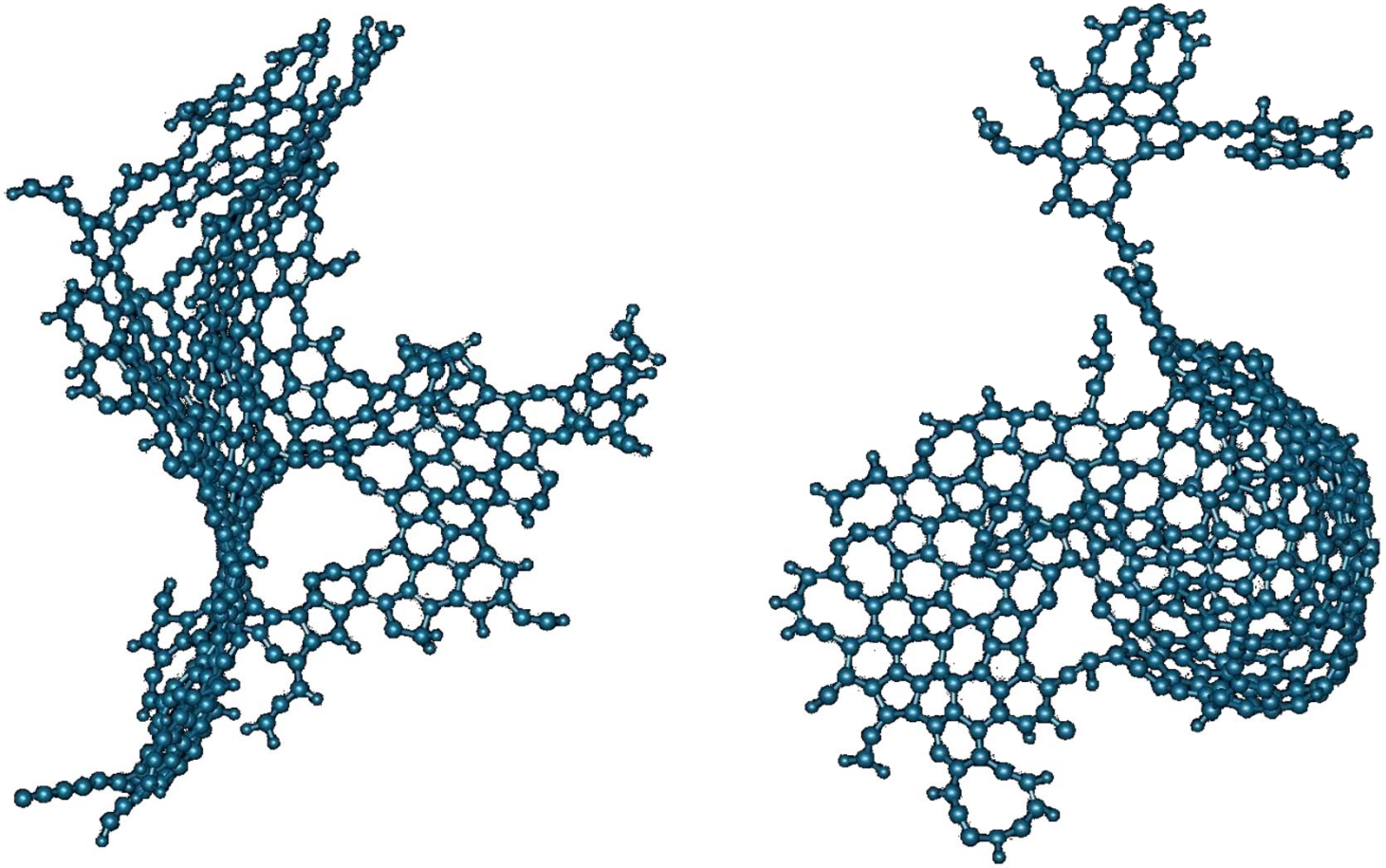
Violi, 2006

A new model for nanoparticle formation

- New computational model that can describe the growth of nanoparticles in high temperature conditions.
- Use of Molecular Dynamics and Monte Carlo methods
- It follows the evolution of chemical and physical properties of particles from small aromatics to 3D structures.

Chung, Violi CARBON 2008
Violi *Combust. Flame 2004*
Violi and Venkanathan, J. Chem. Phys 2006

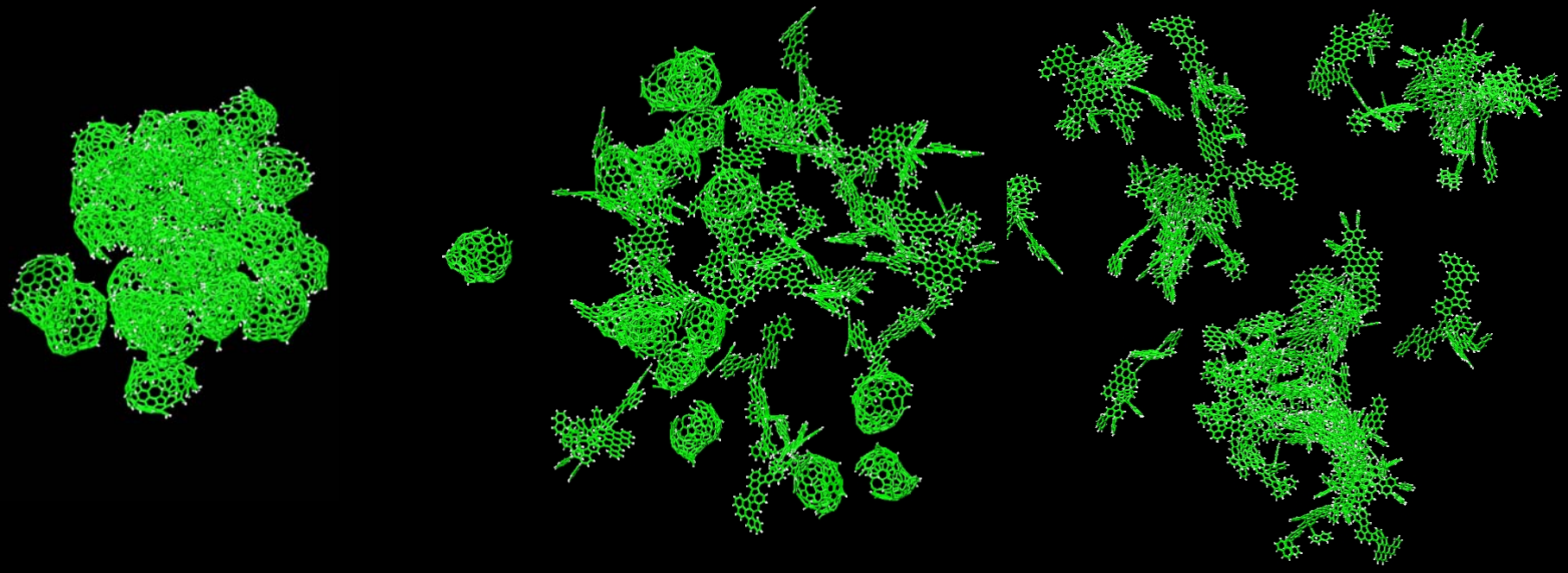
Influence of reaction pathways



Different fuels lead to particles of different morphology and chemical composition

Chung, Violi J. P.ys. Chem. 2010
Chung, Violi CARBON 2008
Violi and Venkanathan, J. Chem. Phys 2006

IMPORTANCE OF MORPHOLOGY



Izvekov, Violi *J. Chem. Theory Comput. 2(3), 504-512 (2006).*

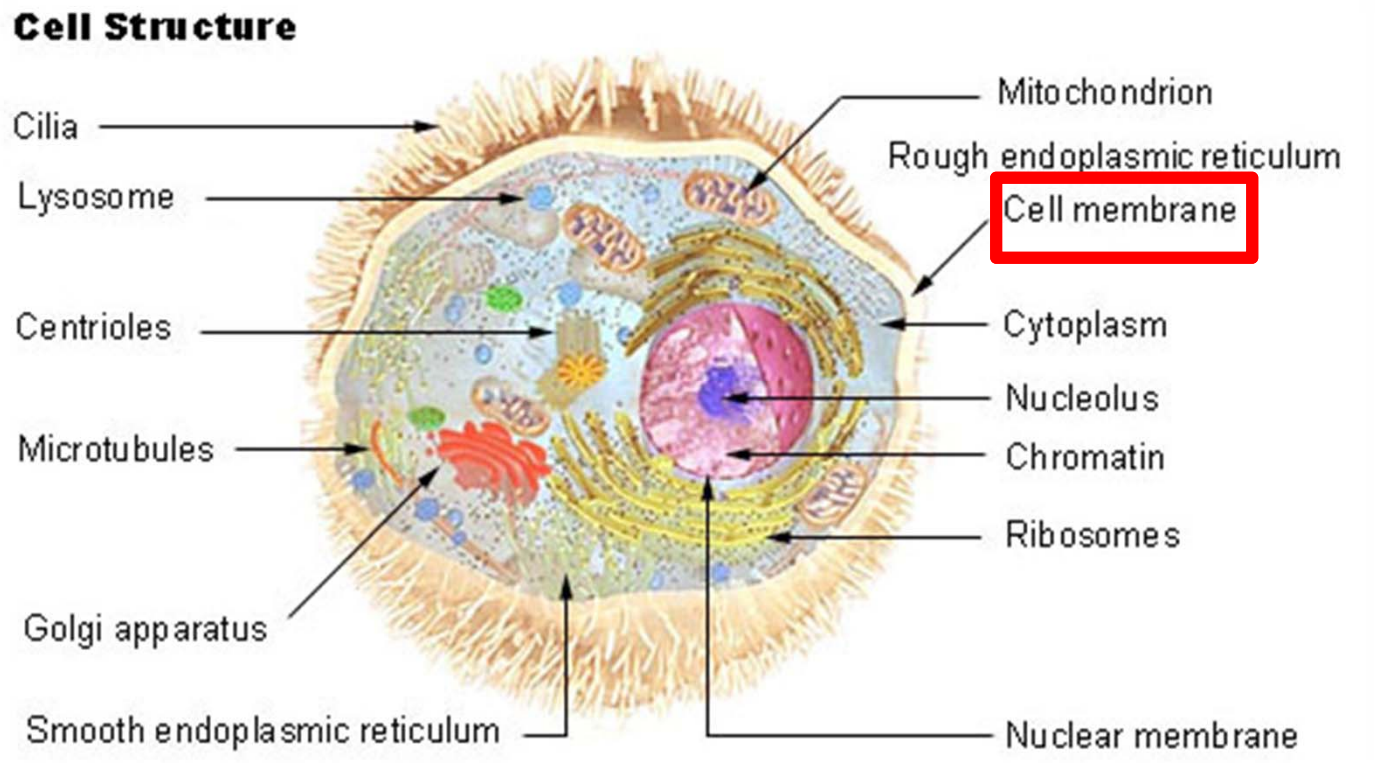
Interactions with Biological Systems

- Uptake of C60 by human macrophage cells and resulting aggregation within the cytoplasm, lysosomes, and the cell nuclei has been visualized using TEM (Porter et ., 2007)

- Appearance of nanomaterial in the cyto- and nucleoplasma of the cell independent of enveloping membrane!

Cells

- There are many different types of cells in the body. However, they all contain three parts: the cell membrane, the nucleus, and the cytoplasm.



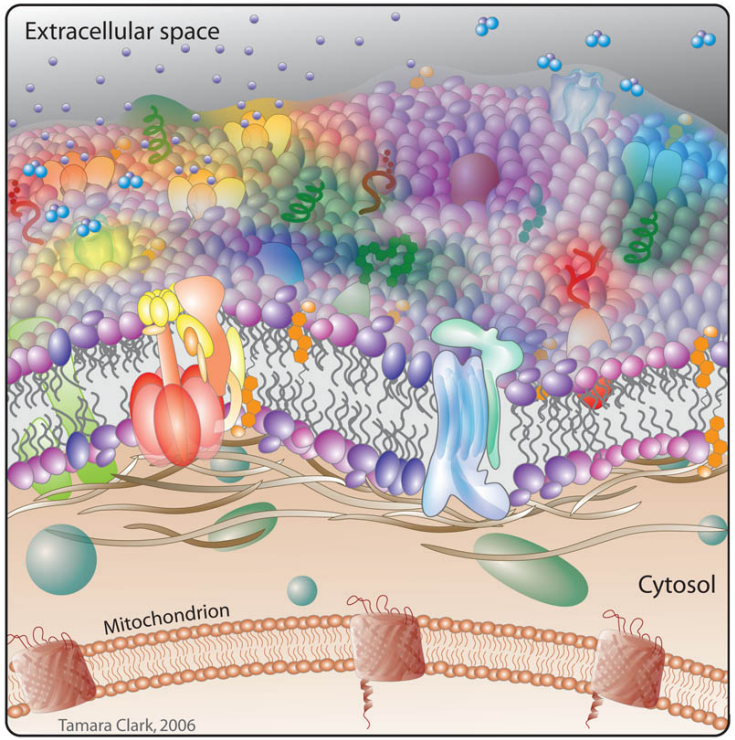
Novelty

- Study of complex membranes with 3 components
- Chol plays important role
- Asymmetric membranes
- New approach to study

the free energy landscape

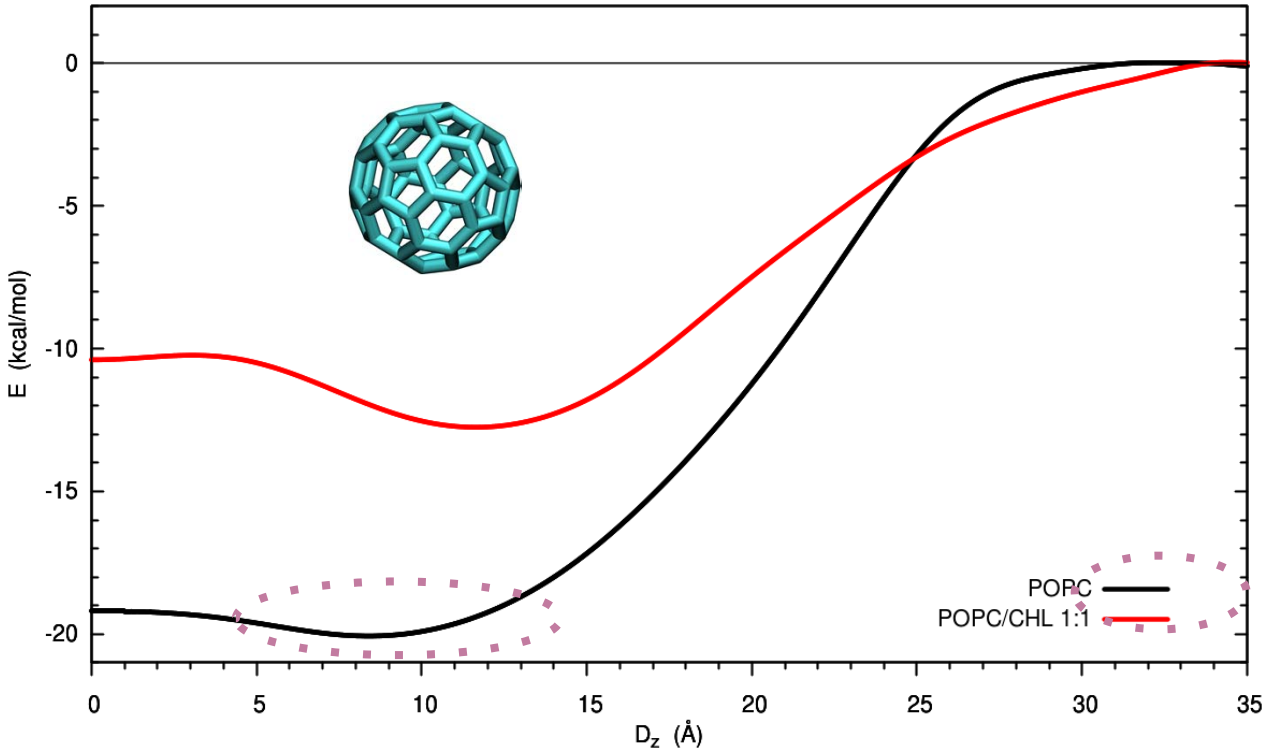
Well-tempered metadynamics

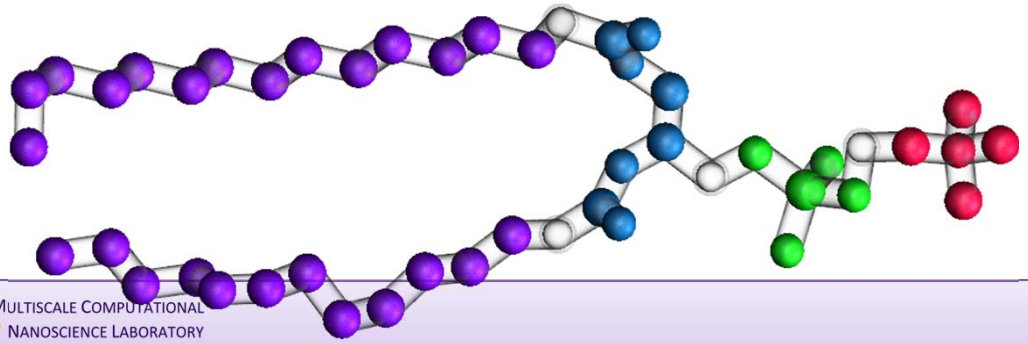
Umbrella sampling



P. Elvati and A. Violi, in *Methods in Nanotoxicity*, in press, J. Reineke, Ed. Humana Press, 2011.

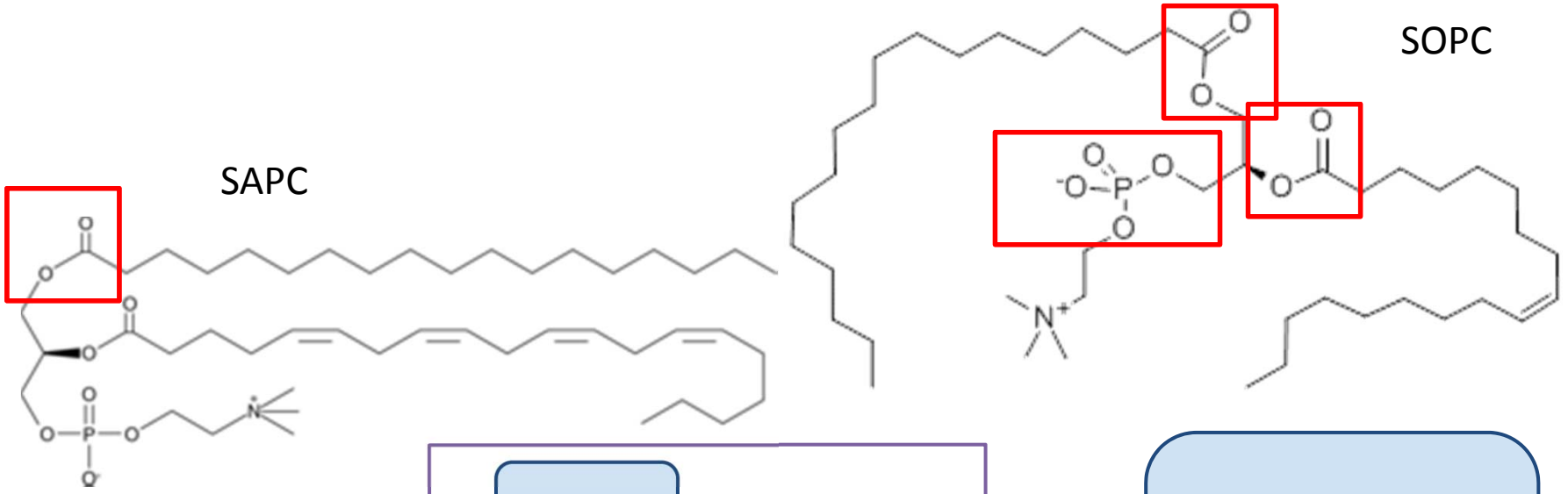
Effect of cholesterol





Mechanism of entry

- Is this a general characteristics?
- Has any influence on the mechanism of entrance?

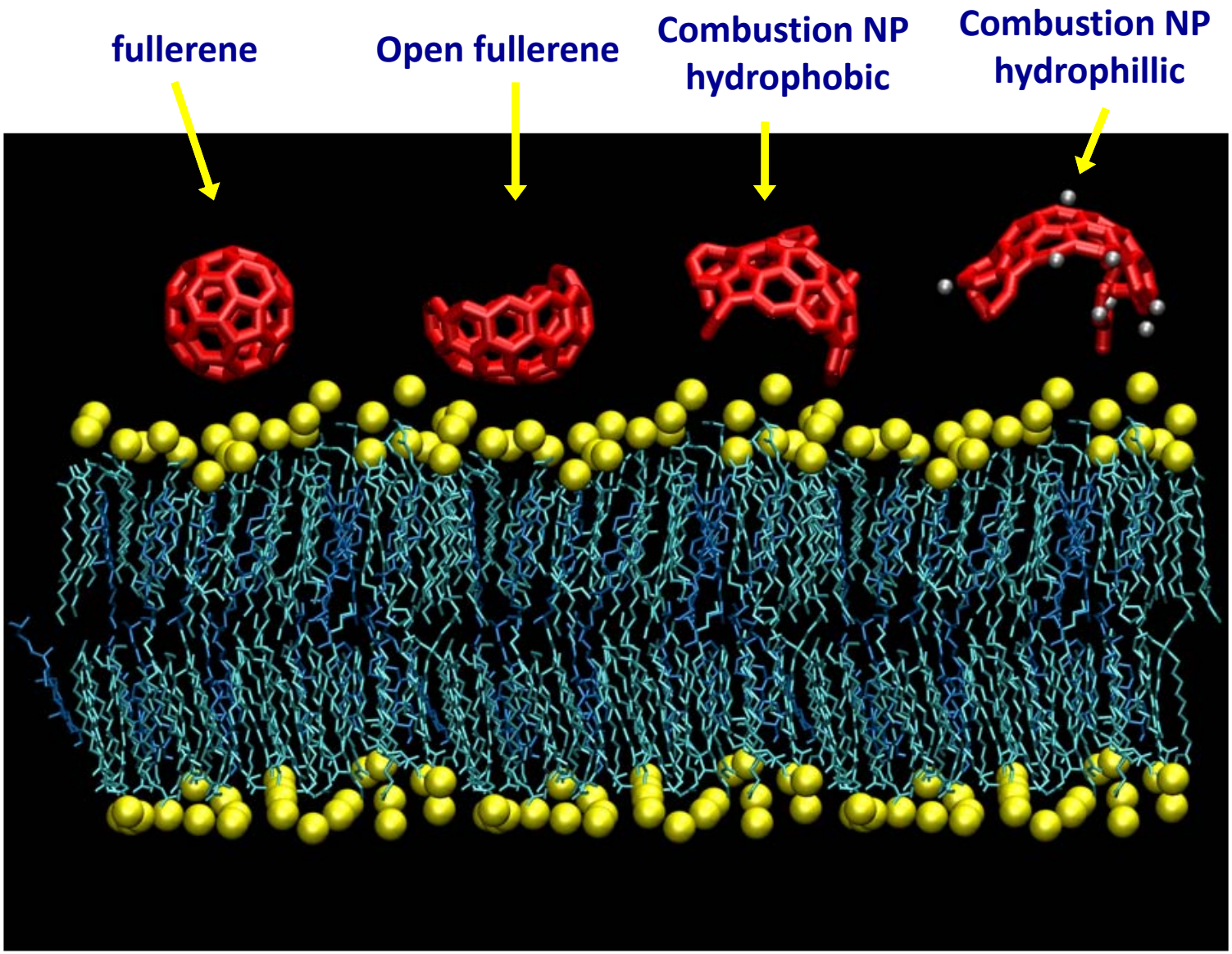


POPC

Cholesterol

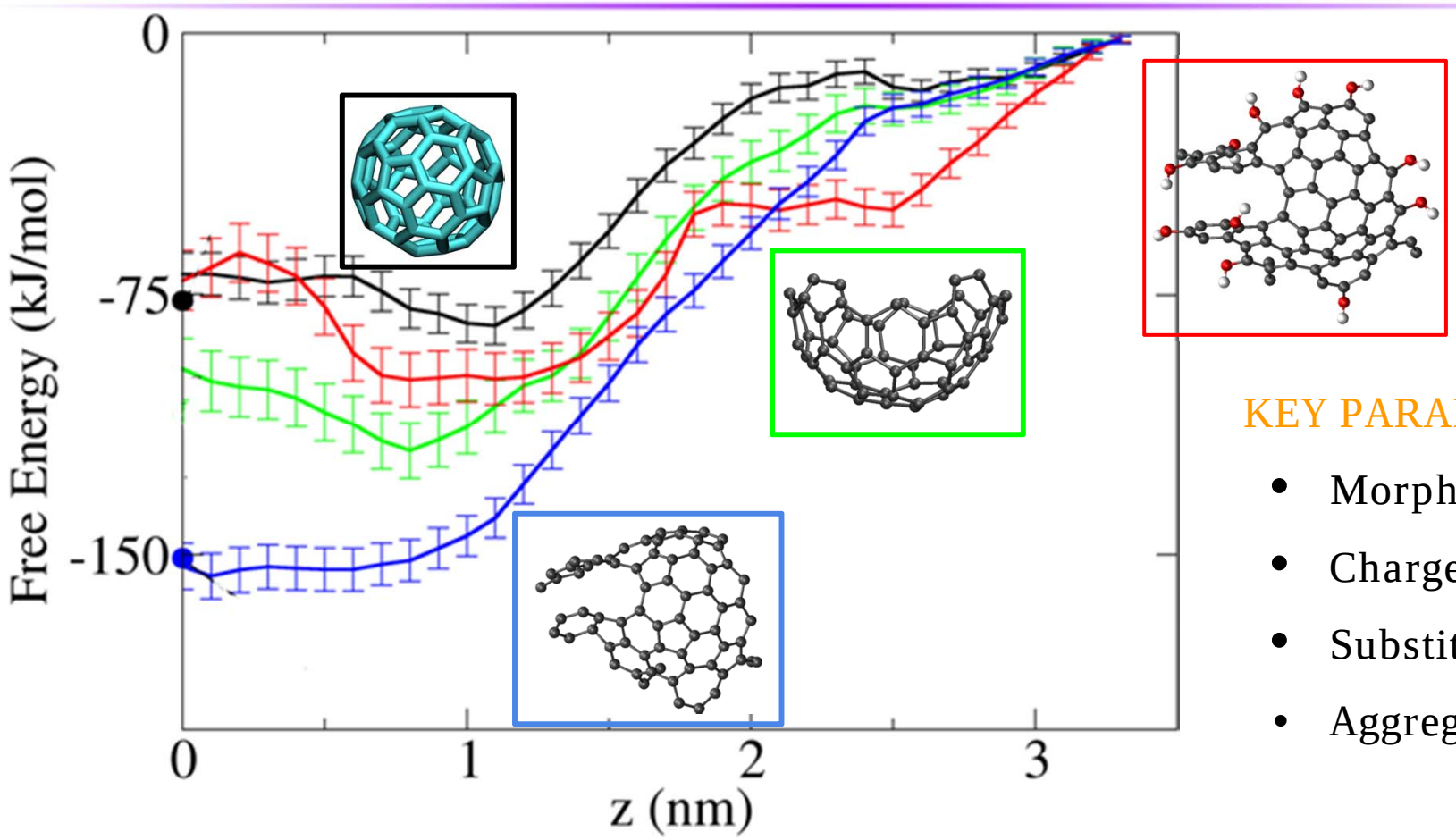
SAPC
SOPC

Different lipid chains (from fully saturated to 4 double bonds)



Fiedler and Violi, Biophysical Journal 99: 144-152 *(2011)*

Permeants



KEY PARAMETERS:

- Morphology
- Charge
- Substituents
- Aggregation

S. L. Fiedler and A. Violi, *Biophysical Journal*, vol. 99, no. 1, pp. 144–152, Jul. 2010.
P. Elvati and A. Violi, in *Methods in Nanotoxicity*, in press, J. Reineke, Ed. Humana Press, 201

Highlights

- C60 is limited in its ability to represent toxicity of similar nanoparticles.
  Need for different dosometric (mass, surface area, volume, number, functionalization, etc.)
- Membrane composition changes the crossing probability and also the mechanisms of interaction.
- Modeling of membrane should move to more realistic systems (permeant characteristics, accurate lipid composition, solvent properties).