# Simulating Carbonaceous Pollutant Nanoparticles
An Aid to Discovery

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Particulate matter (PM) ranks as one of the principal air pollutants linked to acute and chronic physiological ailments. Sized 200 times smaller than a grain of sand, PM is generated by both natural and anthropogenic sources with the latter primarily consisting of fuel combustion processes from utilities, vehicles, and industrial emitters.[1] Since 1987, the U.S. Environmental Protection Agency (EPA) has set an air quality standard for inhalable "coarse" PM sized less than 10 μm (PM_{10}) and in 1997, the agency set stricter standards for "fine" PM, defined to be sized less than 2.5 μm ($PM_{2.5}$).[2] This action was taken to address determinations of greater health risks associated with PM in this category. A subsequent revision in 2006 further highlighted the recognized size-based health risk distinctions by respectively tightening and eliminating the 24-hour $PM_{2.5}$ and annual PM_{10} standards.[3]
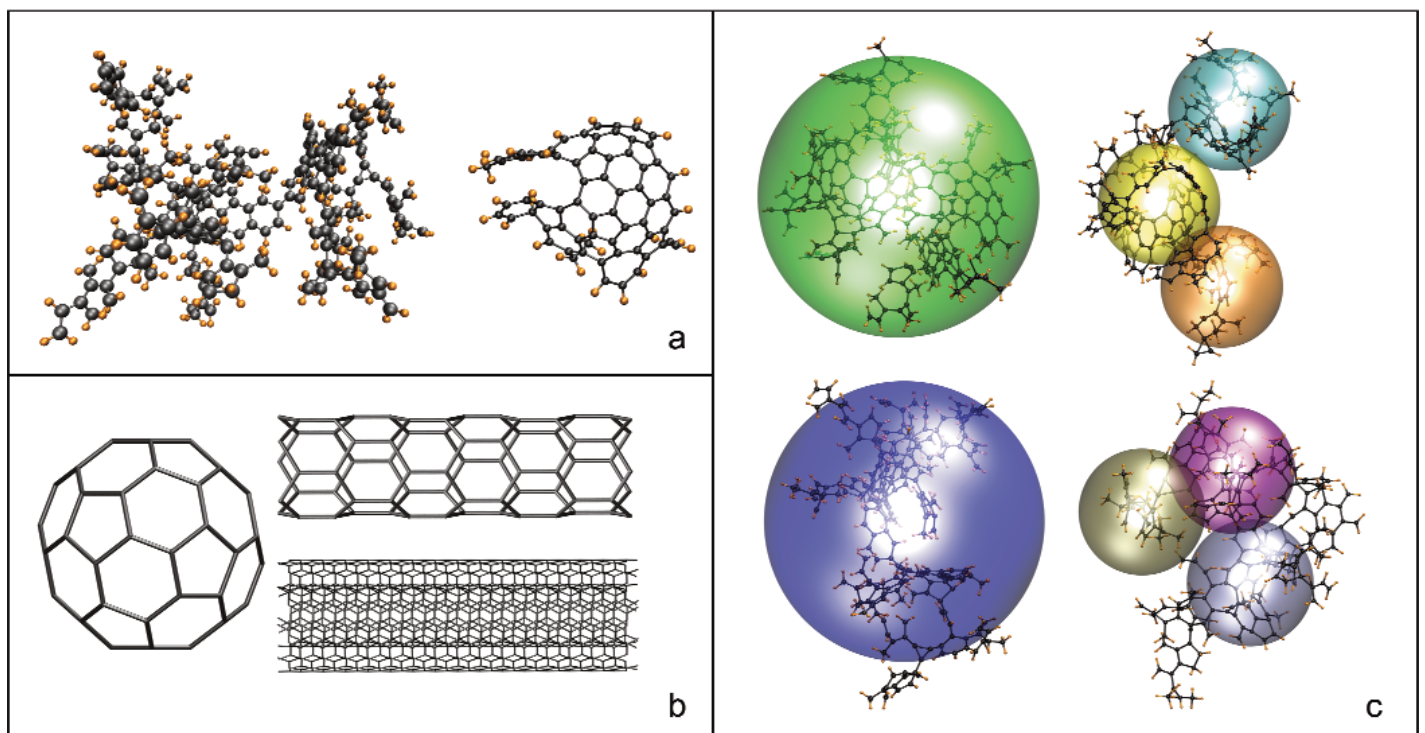
With an understanding of the toxicity of micrometer-sized pollutant particles becoming clearer, a new frontier in epidemiology is emerging with attention directed toward smaller, combustion-generated nanoparticles. Recent studies have indicated a possibility that "ultrafine" particles of less than 0.1 μm ($PM_{0.1}$) may pose an even greater risk than $PM_{2.5}$. Additionally, the aforementioned ambient air quality standards that regulate PM by mass have also been questioned, since in this regard, nanoparticles are not proportionally represented.[4] In fact, it has been estimated that $PM_{0.1}$ account for 80% of the particle number concentration in urban environments.[5] To begin tackling open toxicological questions, this article considers the health effects of combustion-generated nanoparticles, and the contribution of our molecular level insights gained from computational simulations.

Particle Toxicity

Particle toxicity can scale inversely with size. A common example of this phenomenon is carbon black, which is inert in the bulk, but can induce lung injury when reduced to nanoparticle dimensions. Three explanations have been advanced: the net exposed surface area of the material is inversely proportional to particle size (i.e., fewer atoms in the particle interior, which increases the number of reactive sites); smaller sizes could enhance a particle's ability to act as a reducing agent and generate the superoxide radical (O_2^-), a reactive toxic species; and material (chemical) properties and the electronic structure of the particle can change at such small dimensions, which could create reactive sites on the particle.[6] Experimentally, demonstrations of the destructive and perturbative capabilities of certain nanoparticle species have been observed in *in vitro* cytotoxicity studies,[7] and neutron, X-ray scattering, and spectroscopic measurements;[8] however, conclusive determination of the toxicity of even prototypical "simple" nanoparticle species has proven difficult, with buckyball molecules, C_{60}, a case in point.[7]

Figure 1. (a) Representative combustion-generated nanoparticles calculated with the AMPI code suite; (b) Prototypical nano-sized carboneous particles: fullerene (C_{60}), and single- and multi-walled nanotubes currently under EPA review;[10] (c) Coarse graining of combustion-generated nanoparticles into one (left) and three sites (right).

To address concerns regarding public reaction to (im)proper environmental risk assessments, a call for standardization of nanoparticle toxicity measurement techniques has been issued.[9] To this end, an international regulatory consortium, including EPA, specified a set of prototypical nanomaterials for toxicological testing, of which, carboneous molecules are represented by C_{60} and nanotube molecules (see Figure 1b).[10] There is an obvious confluence of concerns of ultrafine pollutant particles and the risk of manufactured nanomaterials. In fact, combustion is the dominant pathway by which anthropogenic particles are emitted into the environment. With this in mind, we have developed a set of approaches to specifically study biochemical interactions of combustion-generated nanoparticles, which differ from these prototypical particles currently under review.

Importance of Modeling

Computer simulation is a standard research and developement tool used to gain a fundamental understanding of macroscopic phenomena and optimize design. Once established, models and simulations can minimize development cost or time and elucidate mechanisms in a manner sometimes unobtainable by experiments (e.g., the correlation between contaminant particle size and toxicity). Systems on the continuum scale are particularly amenable to simulation due in part to the maturity of the methods such as computational fluid dynamics and finite element analysis. Relatively small molecular systems comprised of less than 10–100 atoms can also be simulated with a degree of confidence using quantum mechanical (*ab initio*)-based approaches.

Due to computational cost and the length of time required to run the simulations, larger molecular systems (10^3–10^5 atoms) require more efficient approaches. Often efficiency necessitates a degree of empiricism that tends to scale proportionally with size of the system (number of atoms) or timescale of the behavior simulated. Molecular modeling is a common approach that falls in this category, where intra- and inter-molecular interaction parameters are derived from experiment or *ab initio* calculations.

Many biological, chemical, and material processes, however, are too large to be efficiently simulated
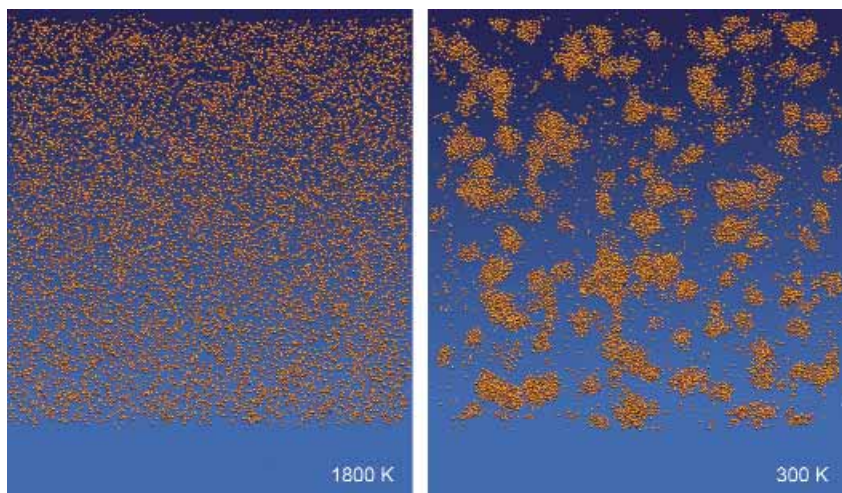
Figure 2. Aggregation of soot precursor nanoparticles at different temperatures. Each orange point represents a nanoparticle of ~400 atoms. As opposed to the disperse distribution of nanoparticles at 1800 K, significant clustering was observed at lower temperatures.
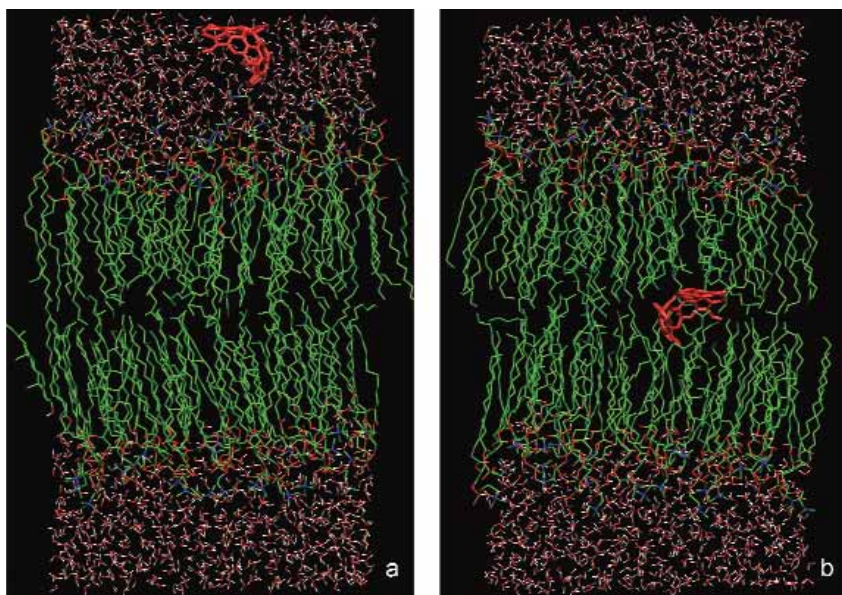

Figure 3. A representative cell membrane in (a) the presence of a carboneous nanoparticle (denoted as "excluded" in Table 1) and (b) and an embedded particle in the bilayer center.

by conventional molecular modeling approaches, but require molecular-level insight that cannot be provided by the continuum-based methods. The multiscale approach developed in the Violi group is designed to fill that void and provide the necessary computational tools to tackle simulation of these mesoscopic-sized systems. Using a hierarchical sequence of methods, a subset of atoms/molecules simulated at high levels of accuracy is used to generate input for the next tier of simulations that incorporate more atoms/molecules or cover longer timescales.

Combustion-Generated Particles

Determining the structures of combustion particles is not a trivial task. Experimental limitations include the fact that removing a particle from an ambient environment for measurement causes deformations. Since the formation of nanoparticle soot precursors spans large time (ps to ms) and length (10^0–10^3 nm) scales and involve large numbers of atoms and molecules, conventional computational approaches are rendered to be too slow to simulate this massive process. A recent computational model developed in the Violi group, termed Atomistic Model Particle Inception (AMPI), combines two standard computational techniques, kinetic Monte Carlo and Molecular Dynamics (MD), to become a tractable alternative.[11-14] The input for the FORTRAN-based AMPI code, include number and concentration profiles of the gas species that contribute to PM growth, reaction rates that govern growth rates, and the temperature profile. The basic premise of the approach is to simulate the growth of a nanoparticle structure in a combustion environment by collisions with polycyclic aromatic hydrocarbons (PAH) and clusters.

Resultant calculated nanoparticle structures, such as those shown in Figure 1a, were found to compare well with known experimental properties.[11,12] There are no inherent size limitations of the nanoparticle "grown" with AMPI; however, computational times vary proportionally to particle size. A calculation for a structure consisting of hundreds of atoms typically requires a couple weeks on modern computational architectures. Presently the AMPI code is proprietary.

We can now also begin to ask questions about the structure and growth mechanisms of larger PM species. It has been hypothesized that an aggregation process occurs for nanoparticles that exceed a minimum size threshold. To consider this process, we employed a multiscalar approach, culminating with the application of a coarse graining procedure, where the interactions of groups of atoms were condensed and represented by larger sites.[15] For computational efficiency, each nanoparticle was represented by a limited number of sites (see Figure 1c), where site–site interaction parameters were obtained from atomic simulations.

Clear demonstrations of the aggregation behavior

were observed (see Figure 2) that could aid experimental structural characterization. Good agreement of the Hamaker constant and the sticking probability of the nanoparticles, was obtained between calculated and spectroscopic values.[15] Using a similar set of computational tools, one could conceivably create designer fuel additives that could shift the PM size distribution outside the range found to be most toxic.

Mechanism of Ultrafine PM Cell Permeation

In a related series of studies we explored the permeation of combustion-generated particles through cell membranes.[16-18] Nanoparticles can enter our system by respiration, ingestion, and through the skin. Since inhalation is presently a primary concern, we considered permeation mechanisms across lung surfactant[17] and epithelial cells lining the respiratory track.[16,18] Immediate clues to permeation mechanisms were apparent in the surfactant study, as surfactant molecules (lipids) were found to wrap around the nanoparticle. Furthermore, the nanoparticle and a representative lung surfactant protein were observed to repel each other.

Interesting insights were also obtained by observation of nanoparticles permeating cell membranes (lipid bilayers). Previous computational studies considered the permeation of small molecules,[19-21] C_{60},[22,23] and nanotubes.[24] Combustion-generated particles though, differ from these and the prototypical, synthetic, nanoparticles currently under toxicological evaluation (see Figures 1a and 1b). To determine the influence of nanoparticle morphology and chemical composition on the diffusion process through the cell, we used a molecular modeling-based approach in conjunction with AMPI.

From the simulations (see Figure 3), the presence of a permeated nanoparticle was found to hinder the motion of the surrounding lipid and cholesterol molecules, effectively acting as an anti-plasticizer in the membrane (see Table 1).[16,18] This instigated hardening has toxicological implications, since some membrane processes such as cell signaling, regulate or depend on membrane fluidity. We can also obtain a microscopic view of mechanisms
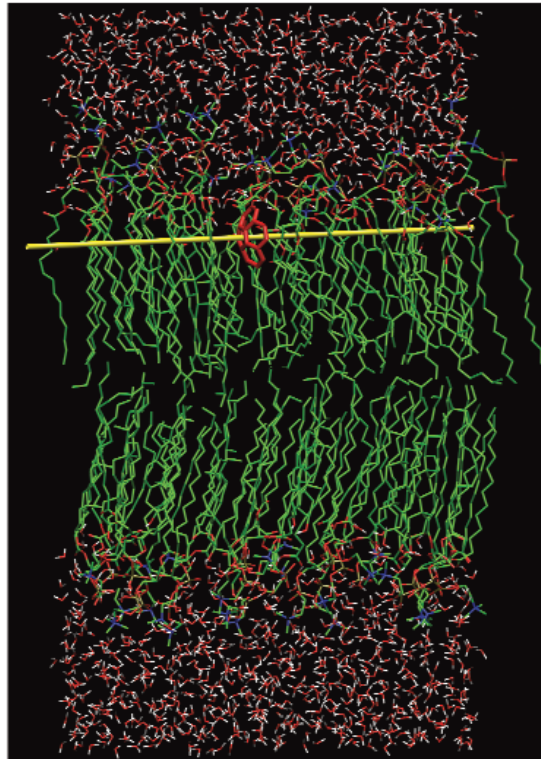

Figure 4. Profile orientation of a corannulene ($C_{20}H_{10}$, 250 amu, 6Å diameter) permeant (red) highlighted by a perpendicular axis (yellow).

associated with the permeation process. As depicted in Figure 4, molecules sized from benzene to the combustion-generated nanoparticle ($C_{68}H_{29}$) were found to orient to minimize their footprint and pass through the membrane with a profile parallel to the bilayer norm. Similar behavior was also previously observed for hexane.[21]

Particle sphericity was shown to be an influential factor for the preferential location that nanoparticles would settle as they passed through the membrane.[16] The particles were not found to be locally trapped however, but could travel at rates in accordance with their molecular masses. The propensity of PAH and nanoparticles to enter and continue to pass though the cell membrane, was also demonstrated from calculated thermodynamic free energy profiles[18] to be highly correlated to the particle surface area.[18] This correlation was not surprising, since as stated above, toxicity has been attributed to a high particle surface area to mass ratio that can enhance chemical reactivity and facilitate its role as oxidant. Quantitative agreement was obtained by comparison of the above free energy profiles to available literature values for benzene[20] and C_{60}.[22]

Table 1. Diffusion constants (10^{-5} nm^2/ps) of constituent molecules in a representative lipid bilayer, dimyristoylphosphatidylcholine (DMPC)/cholesterol (Chol) in the absence and presence of a combustion-generated nanoparticle ($C_{68}H_{29}$; see Figure 3). Embedded and excluded systems are defined as simulations with particles located in the bilayer center and outside the membrane in the water layer, respectively.

System	DMPC	Cholesterol	Nanoparticle
Neat DMPC/Chol	14.4	10.9	N/A
Embedded Nanoparticle	5.2	7.8	6.6
Excluded Nanoparticle	8.8	9.4	19.4

Using computational tools, one could create designer fuel additives that could shift the PM size distribution outside the range found to be most toxic.

Conclusion

By continuing to improve our understanding of biologically active nanoparticles, computational simulations can lend a valuable hand for establishing accurate toxicological assessments. The altered properties possessed by nanoparticles from that of their bulk values complicates the evaluation process. These and other experimental hurdles[8] necessitate a careful, methodical approach to *in vivo* and *in vitro* studies, which can attenuate the convergence of toxicity determinations. Modeling and simulation can provide an economical approach to view toxic mechanisms at the molecular level. Once a base understanding of open questions related to ultrafine particles is achieved, the toxicity of particles in this class can be assigned with a higher degree of confidence. **em**

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