Injecting Carbon Nanostructures in Living Cells

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ABSTRACT

Carbon nanoparticles are currently proposed as reinforcing agents in synthetic biological membranes, able to be embedded in living cells and membrane bilayers. Within a biological environment, porous carbons are anticipated to carry out specific actions, similar to the functionality of known assemblies of biological channels like cyclic peptides and aquaporins. An attainable approach to delve into the mechanism of how carbon pass through the lipid matrices is to use molecular dynamics (MD) simulations. The mechanism consists of different stages, the relative free energies of which may lie far apart in phase space. This induces high energy barriers between the stages, that cannot be crossed in a single simulation. Such observations are addressed through the application of multi-stage workflows, where we utilize explicit sampling schemes in every stage, ranging form grand canonical partitions, for the loading and release of drug substances, to pulling and umbrella sampling simulations, for the dissociation of nanoparticles. The successful development of workflows relies on the encoding of the dependencies between the stages and the tasks and the assurance that data and parameter variables move between the multi - stage components, appropriately. The scope is to use the workflow as a descriptor to train machine learning models for parameter verification and free energy calulation methods for carbon - lipid interfaces.

CCS CONCEPTS

• **Computing methodologies** → **Simulation support systems**; **Molecular simulation**.

KEYWORDS

molecular dynamics, lipid bilayer, porous carbons

1 INTRODUCTION

If porous carbons are to be exploited as drug delivery systems, it is of both fundamental and practical interest to understand how the carbon interface links to the cholesterol supporters of living cells.[3] Carbons may have nanopores of a size comparable to that of endogenous protein channels but mimicking their affinity and transport properties remains challenging.[6] For instance, surface functional groups may have adverse effects on the integrity of the lipid bilayer as they can be toxic.[4, 12, 17]

The entire mechanism of carbon nanoparticles entering into and exiting from the lipid environment awaits consensus.[15] The most well studied approach to delve into such processes is to use molecular dynamics. However, lengthy simulation runs of "brute-force" molecular dynamics, typically on the nanosecond time scale, would be inefficient to capture the long-time scales of typical biological events, which are frequently on the microsecond or millisecond time scale. More important, the dissociation of nanoparticles through interfaces of cosolvents and bilayers obtains high free energy barriers that cannot be explored using conventional sampling methods.[18] This is because, the probability that a spontaneous fluctuation will bring the system on top of the barrier would be vanishingly small.[7, 16]

These challenges can be addressed through application of specialized sampling techniques such as umbrella sampling and adaptive force biasing. Such techniques usually require a predefined number of executions of single computational tasks. A series of advanced sampling techniques can be algorithmically combined in multi - stage workflows, to handle complex and highly demanding computational processes, like those enrolled in bio molecular simulations.[5, 19] Arguably, nowhere is the importance of workflows greater than in biomolecular sciences where the scientific outcome is intricately intertwined with the ability to execute workflows and computational campaigns successfully.[1]

2 METHODOLOGY

We formulate a multi-stage workflow application to encode the entire process in which carbon nanoparticles land on, bind to and translocate through a lipid environment and release a cargo. This can be accomplished in four sequential computing stages. The first stage describes the adsorption simulations of the drug substance into the pores of the nanoparticle. The second stage performs the pulling of the nanoparticle into the bilayer (figure 1). The third stage is where the nanoparticle is embedded into the membrane and the solvation free energies are computed by decoupling the interfacial interactions. The forth stage prescribes a model for the diffusivity, where the cargo substance exits the space of confinement and dissociates to an arbitrary far distance from the nanoparticle. Herefter, we name the different stages of the workflow as *i*) adsorption stage, *ii*) pulling, *iii*) decoupling and *iv*) drug release stage, respectively.

The four stages of the workflow are partitioned in several subtasks (jobs), the development of which, takes place in separate actions. The four stages are then merged in the workflow, i.e., a unified module capable to be executed in a single submission. We use the term "workflow", to express a front-end application handling a four-stage simulation problem robustly, branching decisions during the stages without the need of user interaction. This development entails the encoding of dependencies of the tasks and stages and the assurance that the data and parameter variables move between the components and tasks, appropriately. This is important because most of the tasks in a workflow use dependencies from a different

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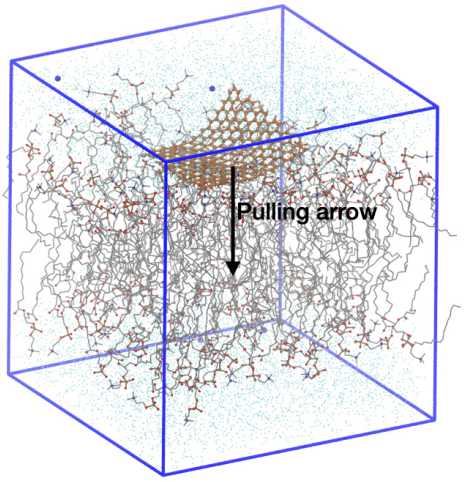

Figure 1: Injecting a single graphene sheet (red hexagonal lattice) into a lipid bilayer. The lipids are shown with gray lines, the hydrophilic head groups of the lipids are shown with red and blue nodes. Water molecules are shown with cyan points, ions in water are shown with blue spheres.

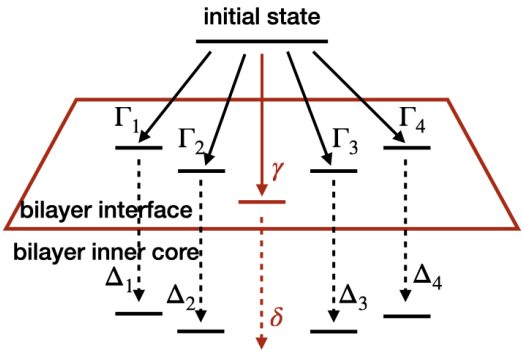

Figure 2: Indicative pathways for nanoparticle injection into a membrane bilayer. We create a single pathway (γ, δ) using pulling simulation and we perform umbrella sampling along the pathway to explore numerous possible trajectories (Γ_i, Δ_i) for the injection. The trajectory inside the bilayer (δ) is sampled repeatedly, in stage *ii* using umbrella simulations and in stage *iii* by decoupling the carbon - lipid interactions, until the estimates for the relative free energy between the two stages statistically converge.

stage and they can be only executed once all of their dependencies have been completed. Although there have been significant advantages in the state-of-the-theory and practice in workflows, the state of workflow development, execution and extension leaves much scope for improvement.

3 POROUS CARBONS

From both chemical and technical perspectives, porous carbons have an important feature; internal cavities. Like in other types of framework materials possessing cavities, substances under confinment are involved in supramolecular interactions, in particular of the host-guest type. To discover, whether one substance can access a specific cavity is a challenging task, because the size and shape of the substance can be very complex. With the nanocarbon model in hand, the only missing component of the theoretical caging prediction is an algorithm that takes the two geometries as input and determines whether the cavity can encapsulate a substance of an arbitrary shape. Algorithms of such type are extensivelly used in the pore size analysis of crystaline porous solids (metal organic and zeolitic imidazolate frameworks), where these solids are evaluated as selective gas filters.[8–10] However, compared to zeolite - type solids, membrane bilayer simulations can depict different, more intricate caging complexes. The time the cargo substance escapes a pore channel, it can be encapsulated by the lipid macromolecules. The lipids configure a cage-like cavity around the drug, that appears like a molecular trap. The trap imposes strong position restraints on the drug, that the simulation should definitely take into account. Within the development of stage *iv* in the workflow (i.e., drug release stage), we employ a revised caging verification algorithm that is able to chemically evaluate and predict the hypothetical formation of lipid - substance (host - guest) molecular complexes.

4 EMBEDDED NANOPARTICLES

Many studies that use molecular simulation to describe the penetration of membrane cells by carbon sorbents, report contradictory results. Some of these studies depict the lipids attached on the carbon surface forming monolayer around the nanoparticle and blocking the pore channels. Different studies report that the lipids are selective to a particular size of nanoparticles, provided that their body is hydrophobic.[13] One side of the nanoparticle has to be shorter than the thickness of the membrane, otherwise the nanoparticle leans in a sideways orientation, in order to maximize its interface contact with the lipids. On the other hand, oxygen containing functional groups at the rim of the pore channels interact with the hydrophilic head groups of the lipid bilayer forming energetically favorable adsoption sites. The functional moieties on the carbon surface, especially the highly polar ones are great contributors of the insertion process. The polar groups affect the potential mean force of the membrane penetration so radically that they may render the membrane impermeable to the nanoparticle. However, in most simulation studies, carbons are initilally embedded in the lipid bilayer without any description of how they have reached that place. Most studies also employ a unified force field,

although it is argued that the surface functional groups should be interpreted with explicit interaction formulas. [2, 20]

We set a reversible path in the *P-T* plane that connects the current simulation system with some reference system of known free energy. This prescription implies the use of pulling simulations (stage *ii*, in the workflow).[11] In pull codes, we apply a constant force spring along a reaction coordinate (path), to gradually displace the nanoparticle from a reference point (point A) to an arbitrary location inside the bilayer (point B), that is the system of interest (figure 2). We compute the derivatives of the free energy on the consecutive steps of this path and integrate. The system of interest may differ from the reference system, not only in its thermodynamic state variables but also in its Hamiltonian. This makes possible a much wide variety of reference systems and reversible paths. This approach is followed in the stage *iii* of the workflow where we make an *alchemica*l change on the system of interest.[14]

In the decoupling stage (stage *iii*), we use point B from the pulling stage as the initial configuration. We remove the nanopartice from the solvent by varying a decoupling parameter $\lambda \in [0, 1]$ in steps of $d\lambda$. The decoupling parameter λ, parameterizes the atomistic interactions between the solvent and the nanoparticle. $\lambda = 0$, corresponds to the state where the interactions are full (point B) and $\lambda = 1$, corresponds to the state where the nanoparticle does not interact with the lipids as if it is simulated in vacuum (point C).[16] This involves the execusion of independent molecular dynamics simulations for the different values of λ. From the simulations we get the average derivative of the parameterised Hamiltonian. Then we compute the free energy, ΔG_{BC}, using integration.

The pulling and decoupling stages use the same system of interest (point B), while their reference systems differ only on the type of solvent, that is water in point A for the pulling stage and vacuum in point C for the decoupling stage. We can compute the difference on the free energy change between points A and C by summing the free energy changes of the two stages, $\Delta G_{AC} = \Delta G_{AB} + \Delta G_{BC}$. The next step is to make a subtle change on an input parameter (i.e., the value of a Lennard jones parameter) and run the pulling and decoupling stages. We change again this value and run this process iteratively, until the free energy change, ΔG_{AC}, converges.

5 CONCLUDING REMARKS

The role of molecular simulation studies are to discover the key factor at the nanoscale which is usually ignored and provide an understanding that will break the conventional way of nanoporous material design and application. Membrane bilayers are complicated molecular systems with several degrees of freedom and correlated torsional terms. In order to sufficiently sample such systems it requires increased computational power and smarter sampling schemes. Using machine learning, we show that the pathway to accurate and reliable methods to compute the free energies of such systems may be clearer than previously thought. This is especially true in the light of new distributed computing techniques, which provide the greatly increased computational power needed for both the development of improved parameter sets and the sufficient sampling of extended ensemble methods.

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