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Hydrophobicity-driven unfolding of Trp-cage encapsulated between graphene sheets

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ABSTRACT

Understanding the interaction between proteins and graphene not only helps elucidate the behaviors of proteins in confined geometries, but is also imperative to the development of a plethora of graphene-based biotechnologies, such as the graphene liquid cell transmission electron microscopy. To discuss the overall geometrical-thermal effects on proteins, we performed molecular dynamics simulations of hydrated Trp-cage miniprotein sandwiched between two graphene sheets and in the bulk environment at the temperatures below and above its unfolding temperature. The structural fluctuations of Trp-cage were characterized using the backbone root mean square displacement and the radius of gyration, from which the free energy landscape of Trp-cage was further constructed. We observed that at both temperatures the confined protein became adsorbed to the graphene surfaces and exhibited unfolded structures. Residue-specific analyses clearly showed the preference for the graphene to interact with the hydrophobic regions of Trp-cage. These results suggested that the conformation space accessible to the protein results from the competition between the thermodynamic driving forces and the geometrical restraints. While confinement usually tends to restrict the conformation of proteins by volume exclusion, it may also induce the unfolding of proteins by hydrophobic interactions.

1. Introduction

The sustenance of life relies critically on the normal biological functions of proteins: maintaining the morphological structures of cells, guiding the transport of nutrients and waste, mediating and regulating cell metabolism, etc. In the cellular context, many proteins perform their functions in confined spaces, such as the chaperonin cavity [1,2] and the ribosome exit tunnel [3], complicated by the geometrical crowding due to neighboring molecules such as carbohydrates, lipids, etc. To understand how proteins function *in vivo*, many previous studies have employed polymer physics models and computer simulations to study the confinement effects on proteins *in vitro* [4–8]. These studies showed that confinement, which imposes volume exclusion, tends to stabilize the compact folded states of proteins by reducing the conformational entropy of the unfolded-state ensemble.

On the other hand, direct experimental visualization of protein structures in solution is usually challenging. In recent years, a rising technique using graphene sheets as liquid cells in transmission electron microscopy (TEM) [9–11] has been applied to image the structural variation of liquid samples, such as the growth of novel nanostructures [9,12–15] and the motions of biomolecules [16–18], with nanoscale resolution. In this technique, the liquid sample, with thickness typically ranging from several nanometers to microns, is commonly sandwiched between two monolayer graphene sheets, protecting the liquid samples from evaporation in vacuum and from excessive local charging and heating induced by the electron beam. Despite the success of graphene liquid cell TEM, it remains unclear how the graphene sheets may perturb the native states of the confined biomolecules. Moreover, as the building block of other carbon allotropes [19], graphene is becoming an important material in biofunctionalization and biotechnologies [20–22]. Therefore, understanding the interaction between graphene and biomolecules is crucial for the design of novel graphene-based biotechnologies.

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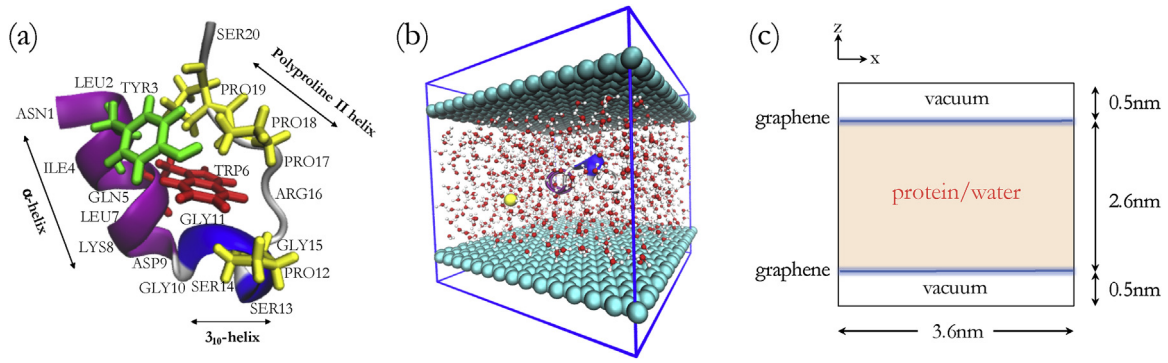

Fig. 1. Trp-cage structure and simulation geometry. (a) Native state of Trp-cage protein. The protein is shown in the new cartoon scheme and colored by its secondary structures: an α-helix (residues 2–8), a 3_{10}-helix (residues 11–14), and a polyproline II helix (residues 17–19). Also shown in licorice are the key residues of the hydrophobic core, including Tyr3 (green), Trp6 (red), Pro12, Pro18 and Pro19 (yellow). (b) A snapshot of the protein-water-graphene simulation box. The cyan spheres represent the carbons of graphene; the red and white spheres represent the oxygen and hydrogen atoms of water; the yellow sphere represents a chloride anion used to balance the charge. The Trp-cage protein is placed in the center between the two graphene sheets before the production MD runs. Two empty spaces are reserved outside the graphene sheets on both sides to avoid overlapping interactions. (c) A schematic illustration of the configuration and the dimensions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Although many computational studies have been devoted to the graphitic carbon–water interactions [23–27], the behavior of proteins under the confinement of graphene has been less explored [28–31]. Furthermore, most previous work mainly studied the protein–graphene interactions at room temperature. To investigate confinement effects in different thermal conditions, in this paper, we used molecular dynamics (MD) simulations to study the structural variations of Trp-cage miniprotein sandwiched between two graphene sheets at temperatures below and above its thermal denaturation temperature. We also studied the protein in the bulk environment for comparison.

Trp-cage (sequence NLYIQWLKDGGPSSGRPPPS) [32] is a designed 20-residue miniprotein with a fast folding time of around 4 μs at room temperature [33,34]. As show in Fig. 1(a), it contains an α-helix (residues 2–8), a 3_{10}-helix (residues 11–14), and a polyproline II helix (residues 17–19). Enclosed by these secondary structure elements is a hydrophobic core constituted by the key residues Tyr3-Trp6-Pro12-Pro18-Pro19 [30,35–37]. These residues form a cage such that the side chain of the residue Trp6 is encapsulated in a sheath of Pro residues. We selected Trp-cage for this work because it has been studied extensively with both simulations [37–40] and experiments [32–34,41–43] due to its structural simplicity and fast folding kinetics. In this work, we quantify the structural rearrangement of Trp-cage using the backbone root-mean-square-deviation (RMSD) from the native state and the backbone radius of gyration (R_g). These quantities together with the simulation snapshots showed that the protein exhibited confinement-induced states when adsorbed to the hydrophobic graphene surfaces. Residue-specific analyses using backbone root-mean-square-fluctuation (RMSF) and solvent accessible surface area (SASA) clearly showed the preference for the graphene to interact with the hydrophobic regions of the protein. Taking the backbone RMSD and R_g as the collective variables, we constructed the free energy landscape of Trp-cage for both the confined and bulk cases. Comparisons across the free energy landscapes of Trp-cage at different geometrical-thermal conditions reveal that the conformation space accessible by the protein is a result of the interplay between thermodynamic driving forces and the influence of confinement interfaces. When the thermodynamic force surpasses the hydrophobic interaction, the conformation space of the protein is mainly affected by the volume exclusion; however, in the opposite scenario, the protein conformation space clearly manifests the presence of hydrophobic interaction between the protein and the confining interfaces.

2. Method

In this work, we carried out MD simulations on hydrated Trp-cage confined between two graphene sheets as well as in the bulk environment. Below we summarize the details of the simulations. The initial configuration of the Trp-cage miniprotein (dimension ~2 nm) was obtained from the NMR structure hosted in the RCSB Protein Data Bank (PDB ID: 1L2Y). For the confined system (see Fig. 1(b) and (c)), a simulation box of 3.6 nm × 3.6 nm × 3.6 nm was constructed with two graphene sheets placed perpendicular to the z direction, leaving a distance of 2.6 nm in between. A Trp-cage miniprotein was placed in the center between the two graphene plates and a chloride ion was added to neutralize the system. The confined space between the graphene sheets was then filled with SPC/E [44] water molecules taken from a bulk water system pre-equilibrated at 300 K. The placement of any two atoms that were closer than the sum of their van der Waals radii were avoided. This procedure leads to a filling of 806 water molecules in the system. Two vacuum volumes were reserved outside the confinement space, isolating the system from interactions coming from periodic boundary condition along the z direction. We simulated the system at 300 K and 500 K, which are below and above the Trp-cage unfolding temperature around 450 K [35,40], respectively. After energy minimization, the confined system was equilibrated in the NVT ensemble at the target temperatures for 10 ns with the protein position restrained to their initial structures. Afterwards, the production MD simulation was carried out in the NVT ensemble for 160 ns to collect the trajectories. The graphene carbons were fixed during the simulations.

For the simulation of the bulk protein system, we removed the two graphene sheets and equilibrated the system in the NPT ensemble at 300 K for 5 ns. This procedure removed the vacuum volumes, leading to an equilibrium system with reduced box sizes, but a similar water density was maintained compared to the confined system. We then brought the system to the target temperatures in the NVT ensemble for 5 ns, and carried out production MD simulation runs for 160 ns.

All simulations were carried out using GROMACS 4.6.7 [45]. The graphene was modeled using the Lennard-Jones parameters developed by Wu and Aluru [27] from random phase approximation, and the SPC/E water model [44] was chosen accordingly as it was the water model used to develop the graphene potential. The protein was modeled using the OPLS-AA force field [46], which has been commonly used in conjunction with SPC water [37,38,46,47]. As SPC/E model is an improved version of SPC model with self-energy

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