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Molecular dynamics simulation of barnacle cement

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Abstract

Barnacle cement is an underwater adhesive that is used for permanent settlement. Its main components are insoluble protein complexes that have not been fully studied. In present article, we chose two proteins of barnacle cement for study, 36-KD protein and Mrcp-100K protein. In order to investigate the characteristic of above two proteins, we introduced the method of molecular modeling. And the simulation package GROMACS was used to simulate the behavior of these proteins.

In this article, before the simulations, we introduce some theories to predict the time scale for polymer relaxation. During the simulation, we mainly focus on two properties of these two proteins: structural stability and adhesive force to substrate. First, we simulate the structural stability of two proteins in water, and then the stability of 36-KD protein in seawater environment is investigated. We find that the stability varies in the different environments. Next, to study adhesive ability of two proteins, we simulate the process of peeling the two proteins from the substrate (graphite). Then, we analyze the main reasons of these results. We find that hydrogen bonds in proteins play an important role in the protein stability. In the process of the peeling, we use Lennard–Jones 12-6 potential to calculate the van der Waals interactions between proteins and substrate.

Keywords: Barnacle cement; Molecular modeling; Molecular dynamics (MD); GROMACS; Structural stability; Adhesive ability; Hydrogen bond; van der Waals interactions

1. Introduction

Some of the strongest adhesives in the animal kingdom are found in marine organisms. One typical example is barnacle, which long intrigued researchers for their fouling activity and their capability for tenacious underwater adhesion to the substratum. Barnacles are exclusive marine animals, and they are the only sessile group of crustaceans. The barnacle achieves the underwater adhesion by secreting proteinaceous substances. Barnacle adhesive proteins, often called cement, are an insoluble protein complex and used for permanent settlement. To adhere effectively, the cement needs to accomplish several functions, such as coagulation, displacement of water from the substratum, establishment of interfacial contact and molecular attraction between dissimilar materials [1]. According to the method of collection, barnacle cements are classified into two types: a primary cement produced while the animal is on a substratum and a secondary cement secreted while the animal is free from substratum [2]. Some reports have indicated that the primary and secondary cements are essentially the same [3].

The biochemical properties of barnacle cement have not been fully elucidated because the insolubility of the cement proteins hampers their purification and characterization. The current study [1–4] provides initial characterization of the major proteins in the cement of barnacles, including molecular weights of separated proteins, amino acid composition analysis, N-terminal sequence and preliminary conformation analysis. The exact results of the cements components, however, are not identical in different reports. So, we just choose two proteins of barnacle cement which have much information such as exact N-terminal sequence for study. One is called 36-KD [4] protein, which has 38 amino acids (Fig. 1). The other one is called Mrcp-100K [1] protein with 28 amino acids (Fig. 2). As far as we know, there are no mechanical properties of barnacle cement, which have been reported, and no report is on describing mechanical behavior using molecular dynamics (MD) simulation method.

Molecular dynamics simulations are based upon numerical solvents of the classical Newtonian equations of motion in which the force exerted on each atom is given by the negative gradient of the potential energy function with respect to the position of the

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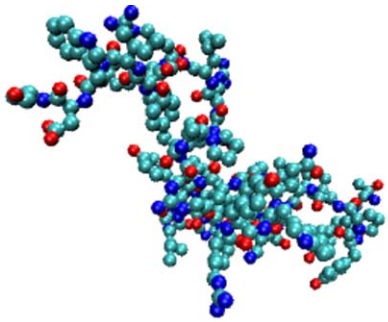
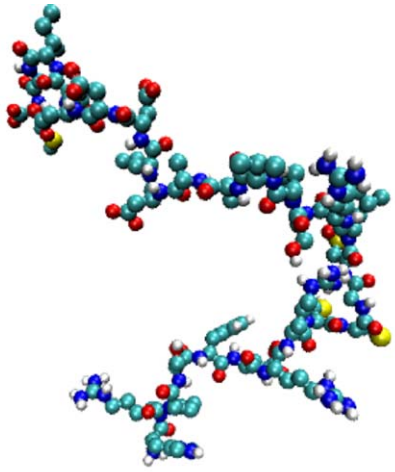


N-terminal sequence: Thr-Tyr-Tyr-Pro-Tyr-Leu-Lys-Thr-Arg-His-Phe-Gly-Gly-Ile-Asp-Leu-Thr-Arg-Tyr-Trp-Gln-Pro-Arg-Tyr-Tyr-Ala-Pro-Ile-Arg-Leu-Arg-Tyr-Pro-Asp-Ile-Trp-Asp-Ala

Fig. 1. 36-KD protein and its amino acids sequence.

atom [5]. GROMACS is a versatile package to perform molecular dynamics, and GROMACS is extremely fast at calculating the non-bonded interactions, so many groups are also using it for research on non-biological systems, e.g. polymers [6]. There is also a wealth of analysis tools and precision-independent trajectory formats.

In this paper, the application of GROMACS for the simulation of structural stability and adhesive ability of two proteins is reported. In our simulation, we are particularly interested in the structural stability and adhesive ability of barnacle cement. We stretched the proteins in different solutions, in water and seawater, respectively. We found that traditional entropic force models are not suitable to describe the behavior of proteins stretching, while hydrogen bonds is essential in keeping proteins' structures. Structural stability of two proteins is almost at the same level in water, but 36-KD protein is more stable in seawater than just in water. And then we peeled proteins from substrate (graphite). We introduced the parameters of van der Waals interaction between graphite and proteins. We found Mrcp-100K protein is more adhesive than 36-KD protein in water. We established some models to analyze the time scale of these simulation processes, and the proteins were deformed over a time scale comparable to the theoretically predicted relaxation time scale.



N-terminal sequence: His-Arg-Pro-Ser-Phe-Glu-arg-Arg-Cys-Cys-Gly-Cys-Leu-Arg-Ser-Pro-Val-Ala-Ala-Asp-Leu-Asp-Asp-Asp-Glu-Ile-Gly-Met

Fig. 2. Mrcp-100K protein and its amino acids sequence.

2. Simulation procedures

The simulation procedures of GROMACS include a series of files and programs. There are mainly three processes. (I) In order to avoid improper structure of proteins, we perform energy minimization at the temperature of absolute zero. (II) Then we increased the temperature of system to 300 K by giving every atom a prime velocity according to Boltzmann distribution, and we balanced the system for a short period of time. (III) We performed the GROMACS package to make sure that the system almost lies in the minimum points of the energy surface, and then we made some simulations, such as stretching proteins to investigate the properties of two proteins. The last frames of each of process (III) are used as starting points for next simulations.

The molecular dynamics simulations were performed using GROMACS Version 3.1. For the proteins, standard GROMACS amino acid residue topology and parameters based on the GROMOS-96 force field were used [7]. The SHAKE algorithm [8] was used to fix hydrogen bonds during the simulation. Leapfrog algorithm was employed for integrating the Newton's equation of motion for each atom, with step of 1 fs. Non-bond pair-list was updated every 10 steps. All the simulations were performed at constant volume and temperature (NVT ensemble) molecular dynamics and the temperatures were always controlled at 300 K, where we used the week coupling scheme of Berendsen et al. [9] to control the temperature.

When stretching the proteins, depending on the dimension of proteins and stretching length, the molecular system was solvated in a periodic 40 Å × 40 Å × 200 Å box with pre-equilibrated TIP3P water molecules as solvent. Na^+ and Cl^- ions were added, respectively, to maintain overall system neutrality. And then we got seawater by adding Na^+ and Cl^- ions to water box with a concentration of about 17%, and the seawater system was also neutral. And when peeling proteins from a graphite surface, the box was defined 70 Å × 70 Å × 120 Å, depending on the dimension of graphite and peeling length. The system was also neutralized by adding Na^+ or Cl^- ions.

The initial structures of proteins simulations were obtained by an equilibration relaxation of 200 ps. We stretched the proteins at the velocity of 0.01 Å/ps. We anchored the proteins on C_α atom of C-terminal of the proteins and imposed a constant velocity on C_α atom of N-terminal of the proteins. All the stretching simulations were performed with the cut-off of 1.5 nm. In the peeling process of proteins from graphite, proteins were placed on graphite with an average separation of about 1 nm, and the system was equilibrated for 200 ps at 300 K. Then, we anchored whole graphite atoms and put a constant velocity on C_α atom of N-terminal of the proteins. The peeling velocity was 0.01 Å/ps with the cut-off of 2 nm. The peeling direction was vertical to graphite substrate.

3. Result and discussion

3.1. Time scale

Time scale is a very important topic in molecular dynamic simulation. The time scales for protein motion and deformation in biological processes may span many orders of magnitude, ranging from fs (10^{-15} s) to a few seconds. Relaxation times are the key parameters of polymer solution dynamics. As to predict the dynamic properties of polymers, polymers should be relaxed after the motion and time controlled features of polymer behavior are correctly rendered, so the motion of a polymer is best described by the longest relaxation time [10]. But there always exist incompatibilities between the time scales of polymer motion and molecular dynamics, so we estimated the time scales of protein stretching and peeling to predict the simulation time.

When stretching the proteins, we first applied polymer dynamic theories to predict the properties of proteins. As we know, Zimm model [11] is very crucial in polymer dynamic theories, so we used polymer's Zimm relaxation time to evaluate the time scale. Relaxation time is the key parameter of polymer dynamics, as it directly correlates with different modes of molecule motion and the hydrodynamic properties of the solution.

In Zimm model, relaxation time is correlative with the length scale of polymer. It is well known that for a chain of N links, the main length scale is the mean squared end-to-end distance

$$\langle R^2(N)\rangle = Nb^2 \sim R^2 \tag{1}$$

where b is the Kuhn length and R is called Flory radius [12]. Each monomer of polymer will undergo a random walk in time, but the whole chain will move as well. The distance of a monomer moves will be large in respect to the monomer size, but small in relation to the polymer molecule as a whole [13],

$$D = \frac{k_B T}{\xi} \tag{2}$$

where D is the diffusion coefficient with dimension $[D] = [L^2T^{-1}]$, k_B the Boltzmann constant, T the absolute temperature and ξ is the Stokes drag coefficient of diffuser given by:

$$\xi(R) \approx 6\pi\eta_0 b N^{1/2} \tag{3}$$

where η_0 is solvent dynamic viscosity coefficient. According to Eqs.(2) and (3), we get Zimm time [11]:

$$\tau_z = \frac{R^2}{D} = \frac{6\pi\eta_0 b^3}{k_B T} N^{3/2} = \frac{6\pi\eta_0}{k_B T} R^3 \tag{4}$$

From the equation of Zimm time, we find the relaxation time scale of a polymer is not only correlative with polymer length scale R, but also relate to the solvent dynamic viscosity coefficient η_0. With $\eta_0 \approx 10^{-3}$ Pa s, $N \approx 10$, $b \approx 1$ nm and $1k_BT = 4.14$ pN nm at 300 K, we expected that a Zimm time of about 10 ns ($\tau_z \sim 10$ ns) used for our simulation. In our simulation, we performed stretching process about 30 ns, which is larger than τ_z.

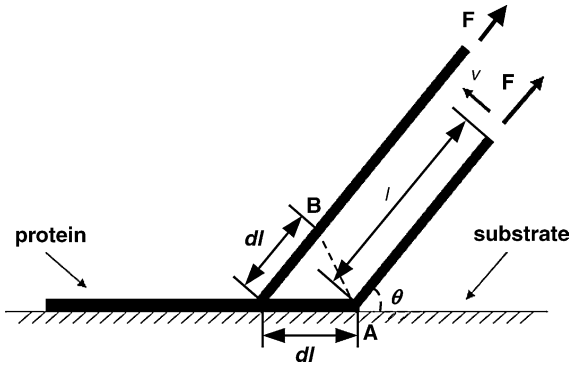


Fig. 3. The peeling model.

In the simulation of peeling process, peeling of a thin film or an elastic band from substrate has been traditionally treated by a linear elastic continuum model [14,15]. The time scale of this model is based on quasi-static condition: compared to adhesion energy and elasticity energy, both the work of solvent viscosity and kinetic energy should be neglected. The total energy of adhesion and elasticity is

$$E = \left(\frac{1}{2}k\varepsilon^2 + \Delta\gamma\right) L \tag{5}$$

where L is total peeling length, k stretch modulus of proteins, $\Delta\gamma$ the adhesive energy per unit length and ε is the strain. As shown in Fig. 3, during the interval dt, distance between points A and B (displacement) is

$$d_{AB} = 2dl \sin\frac{\theta}{2} \tag{6}$$

So the velocity of the peeling end v is

$$v \approx \frac{d_{AB}}{dt} = 2\frac{dl}{dt}\sin\frac{\theta}{2} \tag{7}$$

where dl/dt is peeling velocity and θ is the peeling angle (ε is neglectable). So viscous drag force f of solvent is

$$f = \eta_0 v l \tag{8}$$

where l is the peeled length. According to (7) and (8), we get the work of solvent viscosity with the formula

$$E_v = \int_0^l f\left(2\sin\frac{\theta}{2}\right) dl = 2\eta_0 v \sin\frac{\theta}{2}\int_0^l l\,dl = 2\eta_0 l^2 \frac{dl}{dt}\sin^2\frac{\theta}{2} \tag{9}$$

To neglect the work of solvent viscosity means $E \gg E_v$, so

$$\frac{E}{E_v} = \frac{\left(\frac{1}{2}k\varepsilon^2 + \Delta\gamma\right) L}{\left(2\eta_0 l^2 \sin^2\frac{\theta}{2}\right)\frac{dl}{dt}} \gg 1 \tag{10}$$

So from Eq. (10) we got

$$\tau = \frac{L}{dl/dt} \gg \frac{4\eta_0 l^2 \sin^2\frac{\theta}{2}}{k\varepsilon^2 + 2\Delta\gamma} = \tau_v \tag{11}$$

The kinetic energy E_k is

$$E_k = \frac{1}{2} L\rho v^2 \tag{12}$$

where ρ is line density of proteins. Because E_k should be neglected, so from Eqs. (5) and (7)

$$\frac{E}{E_k} = \frac{\frac{1}{2}k\varepsilon^2 + \Delta\gamma}{2\rho \sin^2\frac{\theta}{2}(dl/dt)^2} \gg 1 \tag{13}$$

and from Eq. (13) we get

$$\tau = \frac{L}{dl/dt} \gg 2L \sin\frac{\theta}{2}\sqrt{\frac{\rho}{k\varepsilon^2 + 2\Delta\gamma}} = \tau_k \tag{14}$$

In the simulation, we estimated that $l \sim L \approx 10$ nm, $k \approx 1000$ pN [16], $\Delta\gamma \approx 100$ pN, $\rho \approx 10^{-10}$ kg/m and $\eta_0 \approx 10^{-3}$ Pa s. According to Eqs (11) and (14), assuming $\sin(\theta/2) \sim 1$ and $\varepsilon \sim 0.1$, we can get

$$\tau \gg \mathrm{Max}(\tau_v, \tau_k) \sim 10^{-11}\ \mathrm{s} \tag{15}$$

where τ is time scale in peeling of proteins. In our simulation, we performed peeling process about 5 ns, which is much larger than τ.

According to Eqs. (11), (14) and (15), τ differs when k and $\Delta\gamma$ are set with different value, which denotes the elastic and adhesive ability of protein, respectively. Also, τ varies with peeling angle θ, which is a parameter of dynamic process. And for τ_v (11), η_0 is a hydrodynamic property of solution, while for τ_k (14), ρ is a bulk property of proteins. Of course, τ becomes longer when the total peeling length L is longer.

3.2. Stretching the proteins

In order to investigate the structural stability of barnacle cement, we stretched the proteins in different environment. First we stretched the 36-KD protein and Mrcp-100K protein in water, respectively.

As shown schematically in Fig. 4, there are mainly two regimes of protein deformation: fluctuation regime and extension regime. In fluctuation regime, resistance to stretching is mostly due to the hydrogen bonds, while in extension regime, the main resistance force is due to bonded interaction.

There're several models of entropic elasticity of polymer stretching, such as the Gaussian chain model, the freely joined chain (FJC) model and the worm-like chain (WLC) model [16–19]. But all these models treat the polymer as a chain of statistically independent segments. It is important to note that these models do not take into account either the presence of a solvent (hydrodynamic effects) or excluded volume interactions. Can these models apply in proteins stretching simulations? According to

$$C = \frac{k_B T}{A} \tag{16}$$

if $F \sim C$ in the beginning of fluctuation regime, we can apply both FJC model and WLC models, where A is the persistence length of the proteins and C is the entropic force of polymer stretching. In our simulation, we assumed $A \sim$ nm [16] and temperature is 300 K, so $C \approx 10$ pN. But in our result (Fig. 5), we can see, in the beginning of fluctuation regime, force $F \sim 100$ pN, so $F \gg C$. In this case, entropic elasticity models of polymer stretching are not accurate enough to describe proteins stretching, because the molecular dynamics simulations implicitly include all of the interact effects of atoms and allow us to analyze chain stretching with excluded volume and hydrodynamics. As we know, hydrogen bonds are important in keeping proteins' structures (Fig. 6). Their average length is 2.5–3.5 Å. In our simulation, we defined hydrogen bond length as 3.5 Å. In Fig. 7, we can see force become bigger when more hydrogen bonds are breaking, and almost every peak of stress curve is a trough of hydrogen bond number curve. For proteins, the average energy needed to break or form a hydrogen bond is about 15–30 kJ/mol. In Fig. 7, we found hydrogen bond energy is an essential cause to affect energy variation of proteins. All of these data indicates that in fluctuation regime hydrogen bonds are more significant than many other interactions, such as entropic force, van der Waals force and so on. And we found structural stabilities of two proteins are almost at the same level in water.

Because barnacle is salt–water organism, we simulated 36-KD protein stretching in seawater. We get NaCl solvent by adding NaCl to water with concentration 17%. Fig. 8 shows that

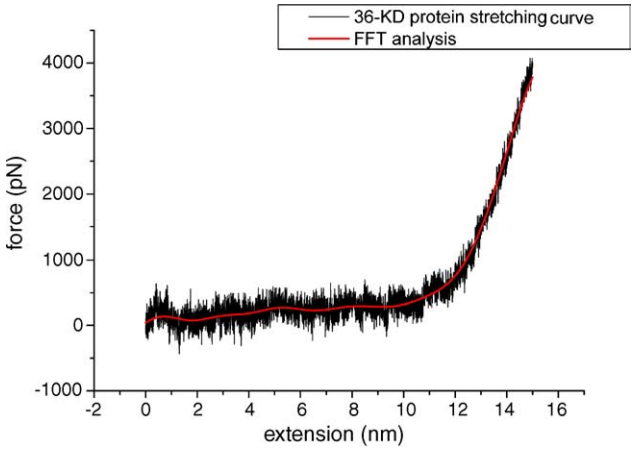


Fig. 4. Extension–force relationship in stretching (in water).

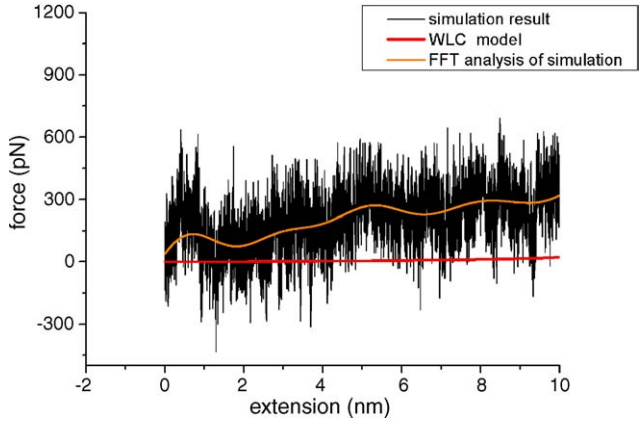


Fig. 5. The fluctuation regime of 36-KD protein stretching (in water).

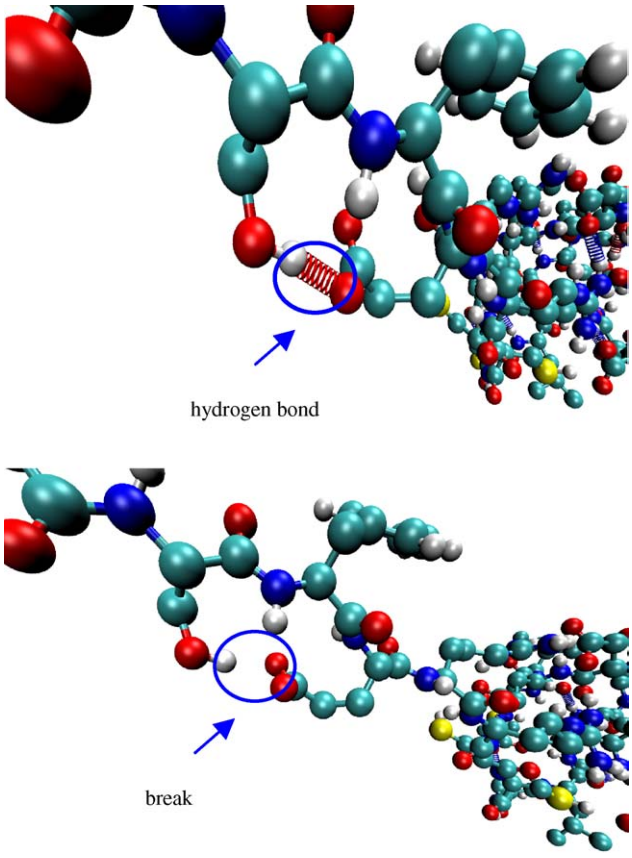


Fig. 6. The breaking of hydrogen bonds.

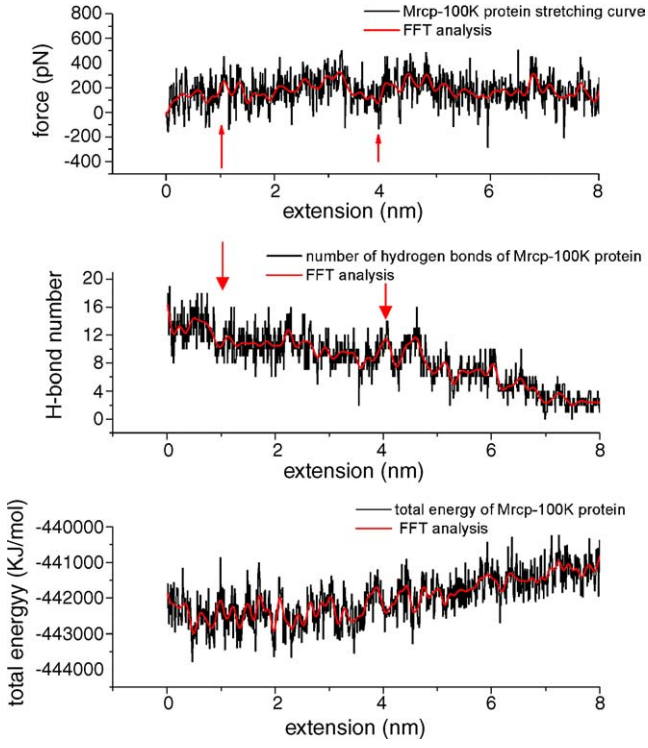


Fig. 7. The variation of hydrogen bonds, strain (stretching) and total energy (stretching).

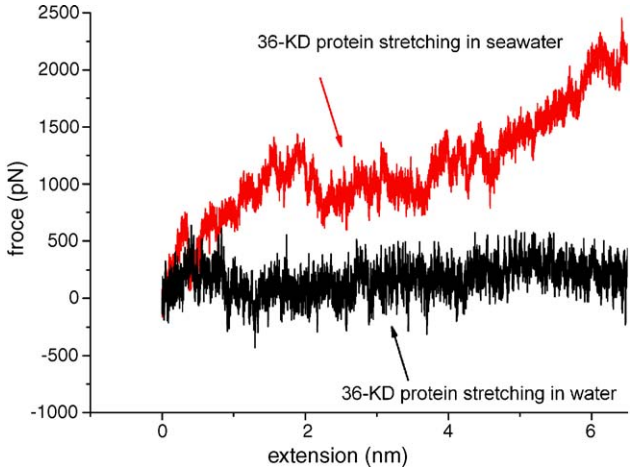


Fig. 8. 36-KD protein stretching in different solution.

force in seawater is larger than that in water. In seawater electrostatic interaction between proteins and ions make the structure of 36-KD protein more stable. In addition, 36-KD protein forms more hydrogen bonds in seawater in fluctuation regime (there are 27 hydrogen bonds in seawater while only 19 hydrogen bonds in water). So 36-KD protein has better structural stability in seawater.

3.3. Peeling proteins from substrate

Because many researchers are interested in barnacle's capability for tenacious underwater adhesion to the substrate, we simulated the peeling process of barnacle cement from substrate.

We choose graphite as substrate, because graphite is inert molecule. It will not have chemical interaction with proteins; graphite cannot form hydrogen bond with proteins and the carbon atom has no charge, so there is no electrostatic interaction between graphite and proteins. Therefore, there are just van der Waals interactions between proteins and graphite substrate. In the simulations, the parameters of force field and the topology files of proteins were based on GROMACS96 force field. But graphite is not biologic molecule, so we got the parameters of graphite from some literature [20]. The non-bond interactions are based on Lennard–Jones 12-6 potential:

$$U(r_{ij}) = 4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right] \tag{17}$$

and the cross interactions are computed using Lorentz–Berthelot combining rules:

$$\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j),\ \varepsilon_{ij} = \sqrt{\varepsilon_i \varepsilon_j} \tag{18}$$

where $\sigma_{cc} = 3.85$ Å and $\varepsilon_{cc} = 0.105$ kcal/mol [21]. The carbon–carbon bond length of 1.42 Å and bond angle of 120° are maintained by a Morse bond, a harmonic cosine angle and a cosine torsional potential [21]. The graphite–water Lennard–Jones parameters are $\sigma_{co} = 3.19$ Å and $\varepsilon_{co} = 0.075$ kcal/mol [20], and those interaction between graphite and protein atoms were

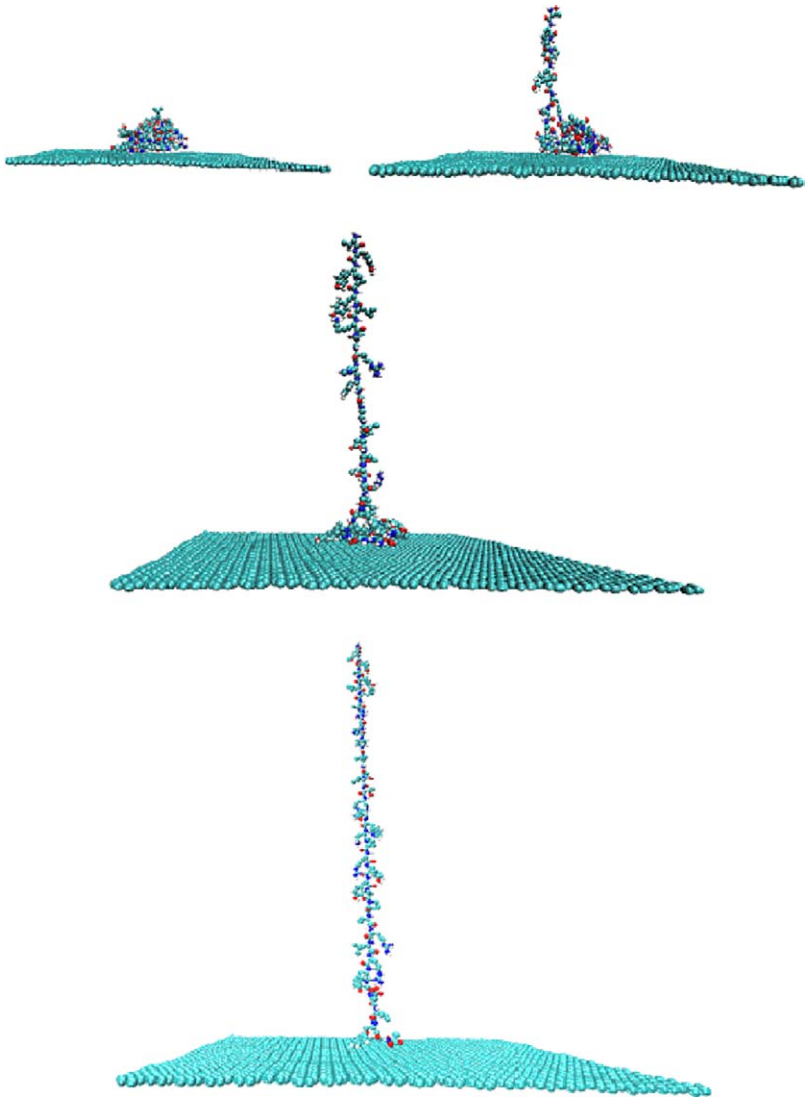


Fig. 9. Peeling protein from substrate (snapshots for 0 ps, 1 ns, 2.5 ns and 5 ns, respectively.).

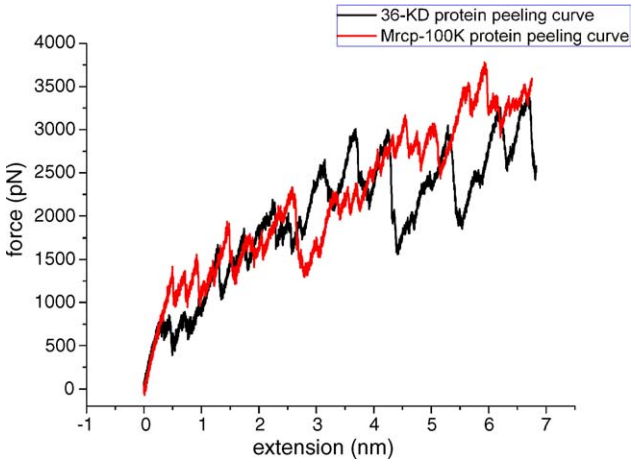


Fig. 10. Extension–force relationship of peeling (in water).

set according to a common used combination rules based on geometrical average [7].

Fig. 9 shows the process of peeling 36-KD protein from substrate. In Fig. 10, we can see that the peeling force of Mrcp-100K protein is almost the same as 36-KD protein. But the peeling process also includes stretching process of proteins. So, the peeling force includes the adhesive force and stretching force. We found that the stretching forces of two proteins are almost the same, so the adhesive forces of two proteins are also close.

4. Conclusions

We found time scale of polymer relaxation is correlated with many parameters including hydrodynamic property of solution and bulk property of proteins. We performed our simulation depending on the time scale of stretching and peeling of proteins we predicted. On the basis of these simulations, we concluded that in barnacle cement, structural stability of two proteins is almost at the same level in water, and proteins are more stable in seawater than in water. Hydrogen bond is important in protein

stretching. And adhesive force of two proteins is almost the same when we peeled the proteins from substrate.

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