NANO-MICRO LETTERS ARTICLE
www.nmletters.org

Cavity Control of Prefoldin Nano Actuator (PNA) by Temperature and pH

Ali Shokuhfar, Ali Ghaffari, Reza Hasanzadeh Ghasemi*

(Received 4 May 2012; accepted 2 July 2012; published online 4 July 2012.)

Abstract: A molecular dynamics study to investigate the cavity control of Prefoldin based bio nano actuator is presented in this paper. Prefoldin is a molecular chaperone with a jellyfish-like structure containing six long coiled-coil tentacles and a large central cavity. We took the temperature and pH of the medium into account, and analyzed the conformational flexibility of the Prefoldin nano actuator in details. Results show that the prefoldin is a very flexible protein, the conformational state of which appears to depend on the temperature and pH values of the medium. In fact, combining these two control parameters, a suitable environment is provided to capture nano cargoes with specific dimensions. These properties of Prefoldin actuator can be used for drug delivery in the body.
Keywords: Prefoldin Nano Actuator (PNA); Cavity control; Molecular Dynamics; GROMACS

Citation: Ali Shokuhfar, Ali Ghaffari and Reza Hasanzadeh Ghasemi, "Cavity Control of Prefoldin Nano Actuator (PNA) by Temperature and pH", Nano-Micro Lett. 4 (2), 110-117 (2012). http://dx.doi.org/10.3786/nml.v4i2.p110-117

Introduction

Recent advances in the field of nanotechnology have enabled widespread opportunities to investigate and manipulate matter on the nanoscale [1]. The research in nanotechnology, combined with important discoveries in molecular biology, has created a new interest in biomolecular machines and robots [2]. In the field of robotics, new initiatives have been proposed to develop nano-scale actuators, joints, motors, and other machine components. These devices will one day be assembled into functional nanorobots [3]. These devices will have intrinsic mobilities that result in their geometry change and hence enable them to perform specific functions [4].

Just as conventional macro-machines are used to develop forces and motions to accomplish specific tasks, bio-nanomachines can be used to manipulate nano-objects, to assemble and fabricate other machines or products and to perform maintenance, repair and inspection operations [5].

In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together they could potentially form nano devices with multiple degrees of freedom, able to apply forces and manipulate objects in the nano scale world, transfer information from the nano to the macro scale world and even travel in a nano scale environment [6].

The most familiar motor is the protein myosin or dynein which moves along filaments, formed through the protein actin, to drive the contraction of muscles. In addition, there are compliance devices such as spring-like proteins called fibronectin, as well as synthetic contractile plant polymers which can act as compliant joints in molecule-size robotic systems [7].

We presented the development of Prefoldin nano actuator (PNA) and their integration in bio nano robotic systems [8]. PNA can be used to manipulate nano-objects.

Prefoldin is a molecular chaperone that is found

Department of Mechanical Engineering, K.N. Toosi University of Technology, #19 Pardiss Alley, Sadra Street, Vanak Square, Tehran, Iran, Zip: 19991 43344, PO Box: 19395-1999
*Corresponding author. E-mail: r_hasanzadeh@dena.kntu.ac.ir

in the eukaryotic cytosol and also in archaea [9-10]. Prefoldin captures a protein-folding intermediate and transfers it to a group II chaperonin for correct folding [11]. In fact, the Prefoldin acts as a transporter molecule that transports bound unfolded target proteins to the chaperonin molecule [12].

Unlike many other molecular chaperones, Prefoldin does not use chemical energy, in the form of Adenosine triphosphate (ATP), to promote protein folding in all cells [13]. Recognition mechanism in archaeal Prefoldin is nonspecific, in eukarya however, Prefoldin has acquired a more specific function [14].

In this manner, Prefoldin performs two mechanical works, 1) capture and 2) transfer, and does not need chemical energy. Unlike eukarya Prefoldin, archaeal Prefoldin is nonspecific. This interesting properties cause, we use archaeal Prefoldin to create a novel nano actuator that can be used for nanoscale manipulation [15-17].

Here, we analyze the conformational flexibility of the PNA in detail, and take the temperature and pH of the medium into account. The results provide valuable information on the flexibility of the PNA when compared to the original structure. Temperature and pH are two control parameters to control of cavity of PNA. In this paper, effects of these parameter on PNA are presented.

Archaeal prefoldin structure

Crystal structure of Prefoldin has shown that Prefoldin has the appearance of a jellyfish with six long coiled coils protruding from it and a large central cavity [18] (Fig. 1). Biochemical and structural studies indicate that these tentacles bind to substrate proteins in a concerted manner and can move outward for efficient interaction with substrate proteins [19, 20]. Each subunit has a hydrophobic groove at the distal region where an unfolded substrate protein is bound. Each tentacle belongs to one of the six Prefoldin subunits, arranged as two subunits (151 amino acids) located in the center of the structure and four of the homologous subunits (117 amino acids) placed at the periphery [21].

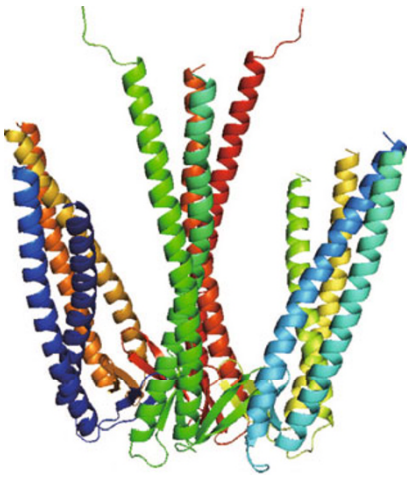


Fig. 1 Overall structure of Archaeal Prefoldin.

Each coiled-coil tentacle is fully solvated. Its polar and charged side chains are almost exposed to the solvent. On the other hand, hydrophobic residues form a hydrophobic groove between the two α-helical regions in the same subunit. These hydrophobic grooves in the cavity are likely to be responsible for the interaction with the hydrophobic surface of an unfolded protein [18].

Molecular Dynamics Simulation of PNA

In this paper, we investigate the effect of temperature and pH on PNA by molecular dynamics. A variety of simulations were prepared to understand the working of the nano actuator under different temperatures and pH.

All MD simulations were performed with the GROMACS 3.3.3 package in which the equations of motion are solved using a leap-frog integration.

The computational model for MD simulations of the nano actuator was generated starting with the crystal structure of the Prefoldin protein obtained from its PDB file [22]. We used the PDB entry 2ZDI, as source for initial state of the nano actuator. To prepare the initial Prefoldin, the DeepView and Pymol software are used.

For all MD simulations, the water cubic box with periodic binding conditions and minimum distance of 1 nm between the solute and the box and with the Tip3p model for water molecules utilizing force field OPLS-AA/L were used. The starting system consists of a simulation box containing one Prefoldin and 38,000-50,000 water molecules.

Energy minimization was performed with steepest descent to release conflicting contacts among residues. The system was equilibrated by 100 ps of MD runs with position restraints on the protein to allow relaxation of the solvent molecules. During the MD simulation, the LINCS algorithm was used to constrain all bonds. A time step of 2 fs was used. The electrostatic interactions were calculated using the particle-mesh Ewald method with a 0.9 nm cutoff. The temperature was kept constant by coupling solute and solvent separately to a thermal bath with a coupling constant dT= 0.1 ps.

Temperature effect on PNA

To investigate the temperature effect on PNA, MD simulation was performed by increasing the temperature gradually from 100 to 330°K to avoid destabilizing the simulated structure. In the Table 1, temperatures and simulation times are presented. Preliminary molec-

Table 1 Temperatures and simulation times of PNA

Temperature (K)	100	150	200	250	273	280	290	300	303	306	308	310
Simulation Time (ps)	75	75	75	75	200	500	500	500	200	300	300	300
Temperature (K)	312	314	316	318	320	322	324	326	328	330		
Simulation Time (ps)	300	300	300	300	300	300	300	300	300	300		

ular dynamics results indicated that by varying the temperature, the conformation of PNA is changed. Structures of PNA at different temperatures are shown in Fig. 2. As it is expected, at low temperature the variations of PNA are very small. But the positions of the arms (tentacles) have drastically changed at high temperatures. The Pymol software package was used as the visualization software for analyzing trajectories generated by GROMACS.

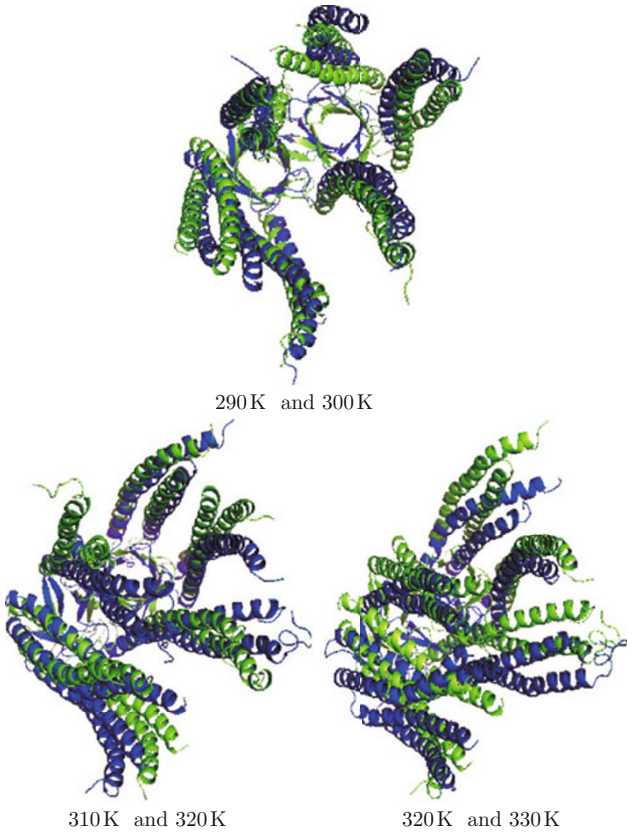


Fig. 2 Conformational changes of nano actuator at various temperatures. Green is lower temperature.

It has been shown that, overall structure of Prefoldin is conserved for all temperatures and shape and size of central cavity has only changed. It is very important in practical applications, such as bio nano robotic systems. The resulting conformational change is due to interaction of Prefoldin subunits with each other as well as with solvent.

Comparison of Prefoldin at different temperatures shows the intense changes of coiled coils. This means that the PNA has dexterity. To capture the nano object by nano actuator, this parameter can be very useful.

pH effect on PNA

A major goal of the present study is the investigation of the pH-dependent conformational changes in the nano object-binding site of the PNA.

We have carried out MD simulations of the PNA at two different protonation states. These protonation states were correlated with the pH of the system; the starting model for this was the Prefoldin protein at neutral pH at different temperatures.

Of the 20 amino acid residues, Histidine, Glutamic acid, and Asparatic acid are protonated at pH range 3–7.4 (acidic pH) depend on their pKa. Prefoldin has Glutamic acid and Asparatic acid, but it has not Histidine. Glutamic and Asparatic acids are initially

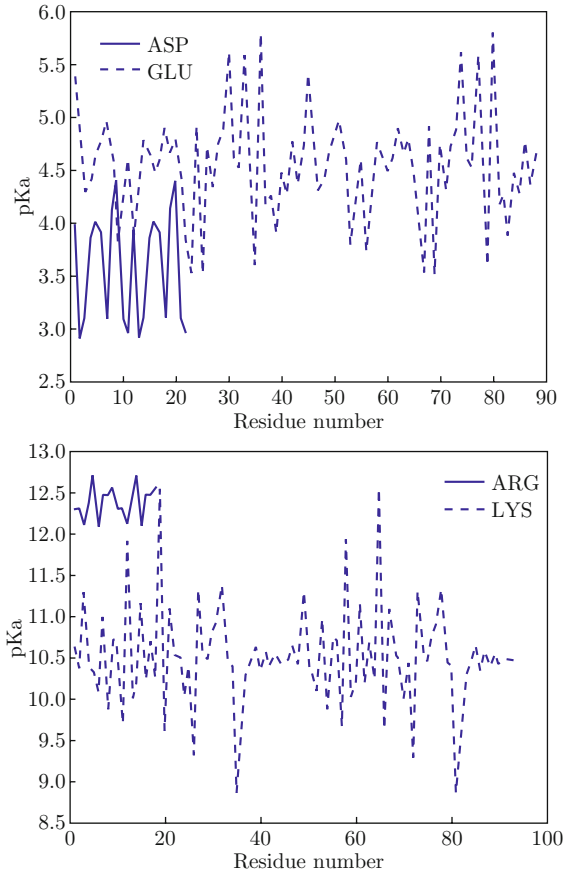
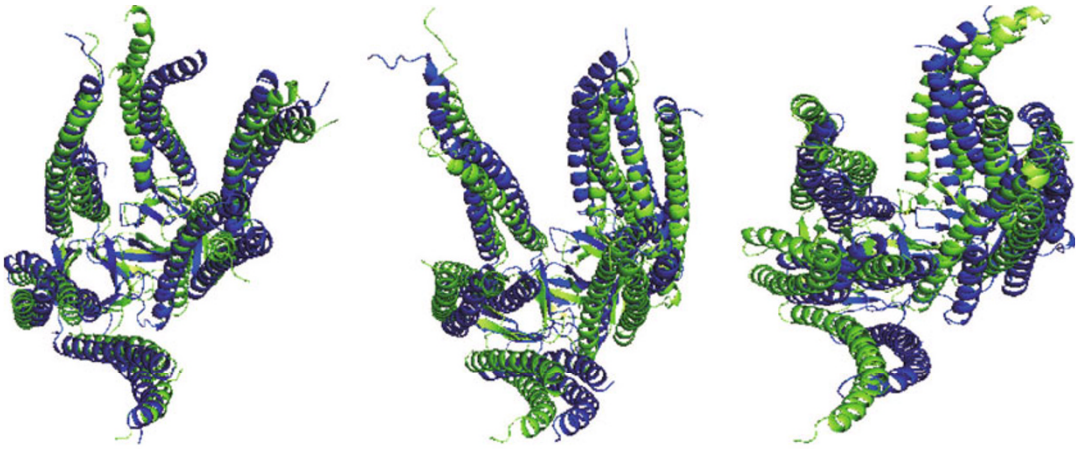


Fig. 3 pKa of special residues determined by PROPKA server.



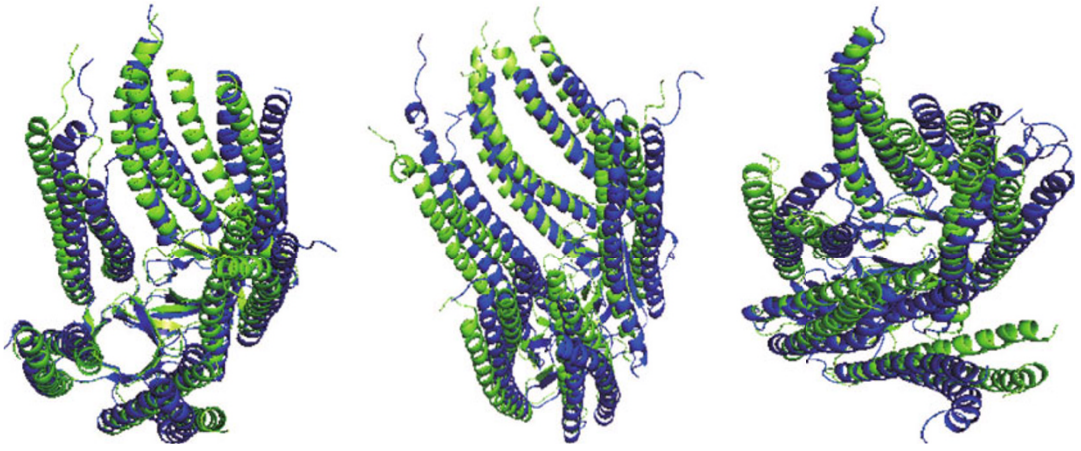


Fig. 4 Conformational changes of nano actuator at various pH. Blue is neutral pH.

negatively charged at neutral pH (∼7.4) and become neutral at lower pH due to the addition of a proton.

At high pH or basic pH (pH range 7.4–13), Lysine and Arginine are unprotonated depend on their pKa. Lysine and Arginine are initially positively charged at neutral pH (∼7.4) and become neutral at higher pH due to the deduction of a proton.

The protonation states of the Prefoldin residues: Asp, Glu, Lys and Arg for the Molecular Dynamics studies were determined in the PROPKA server [23] employing empiric parameters to calculate the pKa of these residues in different environments (Fig. 3).

Fig. 3 shows, All Glutamic acid and Asparatic acid residues are protonated at pH∼3 and all Lysine and Arginine residues are unporotonated at pH∼13. Thus, by the introduction of different Ionizable residues along the peptide chain and varying the pH of the solvent different PNA architectures can be obtained.

To represent a typical biological environment which is electrically neutral, salt (NaCl, 100 mM) was added by placing ions in water to make the system neutral.

In this way, to investigate the pH effect on PNA, 0.5 ns MD simulation was performed. Structures of nano actuator at different pH are shown in Fig. 4.

Area of cavity of Prefoldin: Effect of control parameters on PNA

To accurate studying of PNA, we calculate the area of cavity of Prefoldin observed in each of the nano actuator architecture as a function of temperature and pH from the conformational changes result of the MD simulation. The area is measured as the distance between the Cα atoms of end amino acids of arms (Fig. 5). This

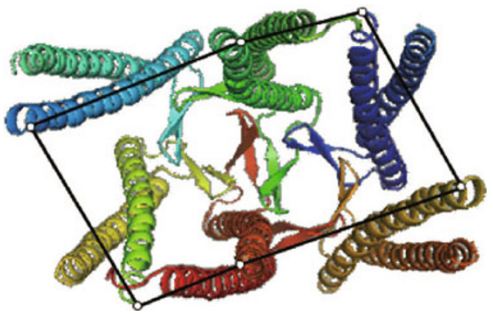


Fig. 5 Area of nano actuator as a variable parameter.

area is a criterion for investigating of cargoes sizes and shapes.

Figure 6(a) and 6(b) show the effect of temperature on this area and change mode of areas, respectively. It is shown that the areas at high temperatures are half of initial area. In fact, as temperature increases, the cavity of PNA becomes closer. This area is also measured for acidic and basic pH (Table 2). These results show, the performance and efficiency of PNA is changed by temperature and pH.

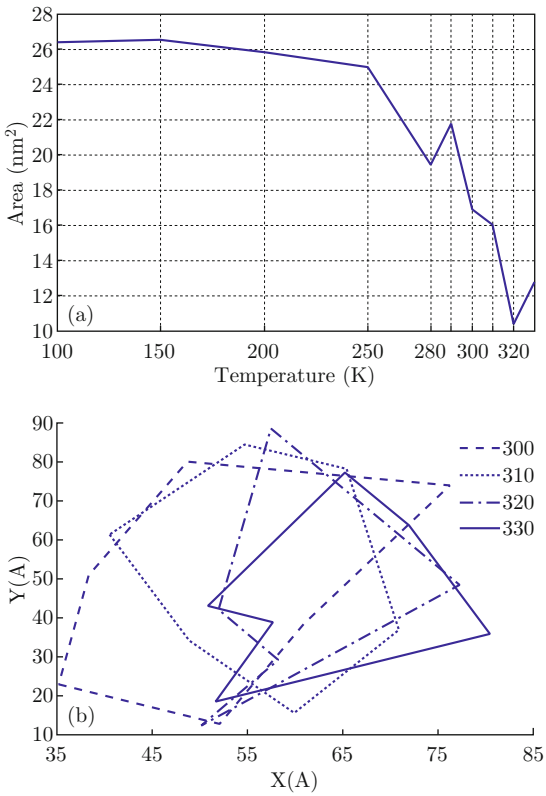


Fig. 6 Area of nano actuator at various temperatures.

Table 2 Area of cavity for three different protonation states (Neutral, Acidic and Basic)

pH	Temperature (K)			
	290	300	310	320
∼3	23.62	20.83	17.80	11.50
∼7.4	21.77	16.82	16.02	10.26
∼13	22.47	16.31	16.23	10.67

The comparison of area of cavity for three different protonation states (Fig. 7) shows, a decrease in pH increases the area of cavity. That is, cavity at acidic pH is more open than neutral pH. At basic pH (high pH), the area of cavity is approximately equal to neutral pH. In other words, increase of pH doesn't affect the performance of PNA.

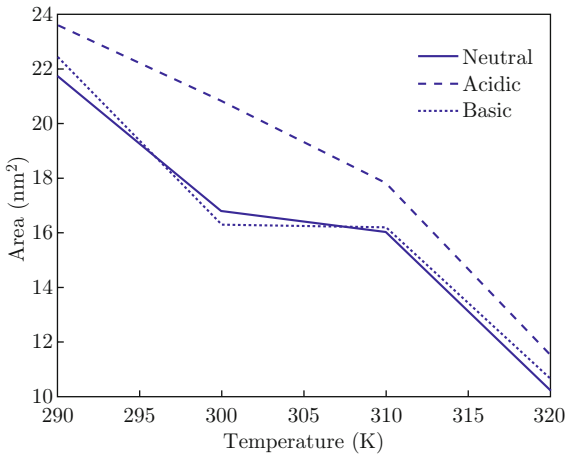


Fig. 7 Comparison of area of cavity for three different protonation states (Neutral, Acidic and Basic).

Discussion

The root-mean-square deviation (RMSD) from the starting structure is an important criterion for the convergence of the system. The RMSDs of the C atoms are shown in Fig. 8, indicating that the whole simulation system appears to have been stable after 300-500 ps of equilibration.

Flexibility of the PNA arms

To identify the most flexible parts of the nano actuator, we calculated RMS fluctuations (RMSFs) for individual residues, and compared them with the crystallographic results.

Figure 9 holds the plots of the RMSFs of Prefoldin simulations derived from the MD trajectory at different conditions.

The RMS profiles reveal the most flexible parts of the nano actuator, which are the ends of coiled coil and regions between the α-helical and β-assembly regions. Also, the regions in the middle of α-helical region are flexible.

The change mode of cavity

The results show that, increase of temperature causes, distance between $\beta 1$ and $\beta 2'$ and distance between $\beta 2$ and $\beta 1'$ are reduced. Distance between $\beta 2$ and α, and distance between $\beta 2'$ and α', as well as distance between α and α' are approximately constant. Reducing the distance between $\beta 1$ and α, and distance between $\beta 1'$ and α'a re intense. Although relative distance between $\beta 2$ and $\beta 2'$ is constant, distance between $\beta 1$ and $\beta 1'$ is reduced very intense.

These observations show that PNA has a rotational motion during molecular simulations. This result is confirmed by Fig. 6(b). Rotational motion can be very interesting in nano scale systems.

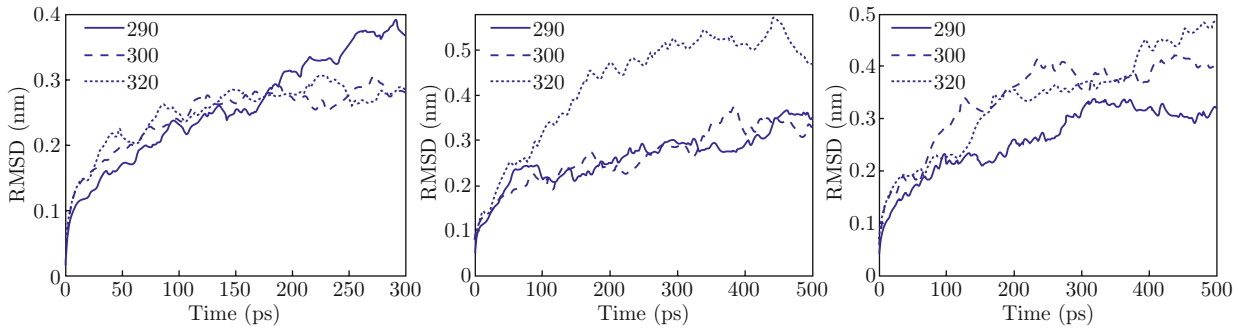


Fig. 8 Time dependence of the RMSDs from the starting structure of the PNA for C atoms during the MD simulation (from left to right: Neutral, Acidic and Basic).

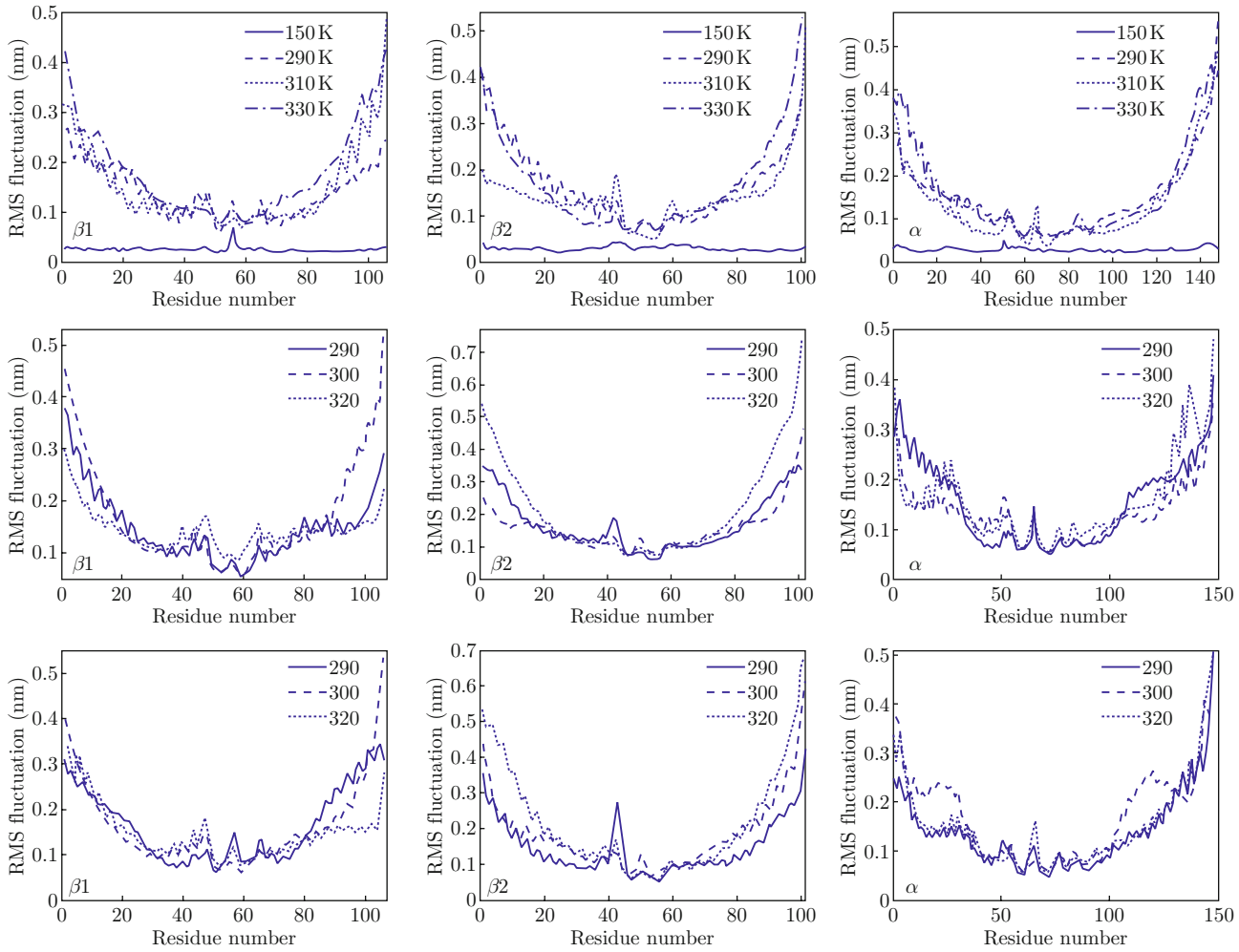


Fig. 9 Root Mean Square Fluctuation of PNA during molecular simulations (from left to right: Neutral, Acidic and Basic).

Effect of flexible tentacle on cavity

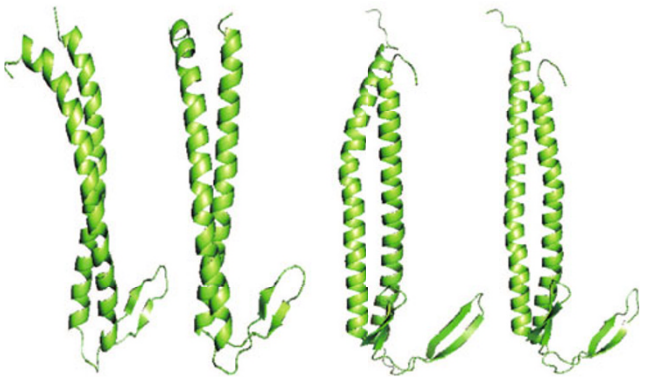
Any arm of PNA is a coiled coil; therefore Prefoldin consists of six coiled coils which were engineered to obtain an environmentally responsive nano actuator involving the movement of arms towards and away from each other.

One important characteristic observed in this nano actuator is the conserved α-helices of the six coiled coil even in the presence of high temperatures and various pH (Fig. 10). This is due to stabilizing effects of the hydrophobic interactions and hydrogen bonds.

Two elements in flexible arms can change cavity of PNA and adapt it to capture various nano objects (see Fig. 10). The first at the base of each arm serves as a flexible joint and produces large changes. Second ele-

ment is the changes produced in arms themselves, that contains smaller changes and can more accurately adjust nano actuator.

(a) $\beta 1'$



(b) $\beta 2'$ (c) α'

Fig. 10 The subunits of Prefoldin nano actuator. (a) Initial Prefoldin and nano actuator at temperature 330 K and neutral pH. (b) Nano actuator at temperature 320 K, Neutral pH and Acidic pH. (c) Nano actuator at temperature 300 K, Neutral pH and Basic pH.

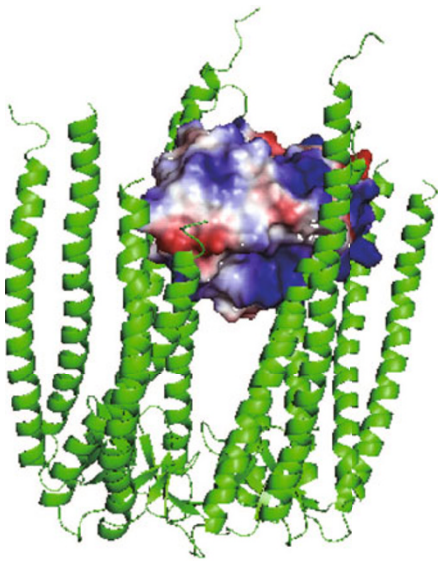


Fig. 11 The schematic of PNA.

In other words, there are two motions in this nano actuator: large and small motions. The large motions cause to open and close the cavity of nano actuator and the small motions adapt the nano actuator with nano objects accurately. As a result, PNA has several degrees of freedom.

In PNA, it is possible to obtain asymmetric changes, namely if a nano object is non regular, nano actuator is able to adapt itself to capture it.

In fact, this nano actuator has interesting properties. These properties are very useful in bio nano robotic systems, because these various changes empower the nano actuator to capture and transfer the size of different nano objects and nano drugs. A schematic of a PNA, showing the working principle, is presented in Fig. 11.

Conclusion

In this study, the dynamics and functions of the PNA is investigated at differnent temperature and pH with MD simulations. According to the MD trajectories, it is found that PNA is capable of exhibiting largescale conformational changes. Particularly, clear opening–closing motions are observed in Prefoldin simulations. Characterized by their remarkably large flexibilities, it is found that the cavity of PNA can change at different conditions.

In this paper, we focused on the mechanical properties of PNA to change its conformation depending on the temperature and pH of environment. During molecular dynamics simulation, each arm (coiled coil) is highly flexible, enabling it to widen its central cavity and capture various cargoes. Our obtained results show how PNA may well be adapted to interact with many different nano objects. Therefore, PNA has a special structure that it can grasp different cargos.

In fact, combining the two control parameters (temperature and pH), a proper environment is provided to capture nano cargoes with specific dimensions. This renders a set of parameters which can be used to compare different conditions and to select the one with the best possible performance results.

References

[1] G. Sharma, K. Rege, C. Mavroidis1 and M. L. Yarmush, “Design and Modeling of a Peptide Based Nanotweezer”, ASME International Design Engineering Technical Conferences, Philadelphia, Pennsylvania, USA, 2006.

[2] A. Dubey, G. Sharma, C. Mavroidis, M. S. Tomassone, K. Nikitczuk and M. L. Yarmush, J. Comput. Theor. Nanosci. 1, 18 (2004). http://dx.doi.org/10.1166/jctn.2003.003

[3] A. Dubey, C. Mavroidis and M. S. Tomassone, J. Comput. Theor. Nanosci. 3, 885 (2006). http://dx.doi.org/10.1166/jctn.2006.005

[4] G. S. Chirikjian, K. Kazerounian and C. Mavroidis, J. Mech. Design 127, 695 (2005). http://dx.doi.org/10.1115/1.1901706

[5] A. Dubey, G. Sharma, C. Mavroidis, S. M. Tomassone, K. Nikitczuk and M. L. Yarmush, "Dynamics and Kinematics of Viral Protein Linear Nano-Actuators for Bio-Nano Robotic Systems", Proceedings of the IEEE, International Conference on Robotics & Automation, New Orleans, LA, April 2004.

[6] G. Sharma, M. Badescu, A. Dubey, C. Mavroidis, S. M. Tomassone and M. L. Yarmush, Transactions of the ASME 127, 718 (2005). http://dx.doi.org/10.1115/1.1900751

[7] M. Hamdi, A. Ferreira, G. Sharma and C. Mavroidis, Microelectronics J. 39, 190 (2008). http://dx.doi.org/10.1016/j.mejo.2006.12.003

[8] A. Ghaffari, A. Shokuhfar and R. Hasanzadeh Ghasemi, J. Comput. Theor. Nanosci. 8, 2406 (2011). http://dx.doi.org/10.1166/jctn.2011.1929

[9] S. Geissler, K. Siegers and E. Schiebel, EMBO J. 17, 952 (1998). http://dx.doi.org/10.1093/emboj/17.4.952

[10] I. E. Vainberg, S. A. Lewis, H. Rommelaere, C. Ampe, J. Vandekerckhove, H. L. Klein and N. J. Cowan, Cell 93, 863 (1998). http://dx.doi.org/10.1016/S0092-8674(00)81446-4

[11] T. Zako, Y. Murase, R. Iizuka, T. Yoshida, T. Kanzaki and N. Ide, J. Mol. Biol. 364, 110 (2006). http://dx.doi.org/10.1016/j.jmb.2006.08.088

[12] http://en.wikipedia.org/wiki/Prefoldin

[13] A. Pockley, Molecular Chaperones and Cell Signaling, Cambridge University Press, Cambridge 2005.

[14] J. Martin-Benito, J. Gomez-Reino, P. C. Stirling, V. F. Lundin, P. Gomez-Puertas and J. Boskovic, Structure 15, 101 (2007). http://dx.doi.org/10.1016/j.str.2006.11.006

[15] A. Ghaffari, A. Shokuhfar and R. Hasanzadeh Ghasemi, "Characterization of Prefoldin Based Nano Gripper", International Congress on Nanoscience and Nanotechnology, Shiraz, Iran, 2010.

[16] A. Ghaffari, R. Hasanzadeh Ghasemi and A. Shokuhfar, "Molecular Dynamics Studies of a Novel Nano Actuator Based on Archaeal Prefoldin", The 17th Iranian Conference on Biomedical Engineering, Isfahan, Iran, 2010.

[17] A. Ghaffari, A. Shokuhfar, R. Hasanzadeh Ghasemi, "Design and Simulation of a Novel Bio Nano Actuator by Prefoldin", IEEE Nano Conference, Seoul, South Korea, 2010. http://dx.doi.org/10.1109/NANO.2010.5697837

[18] A. Ohtaki, H. Kida, Y. Miyata1, N. Ide1, A. Yonezawa1, T. Arakawa1, R. Iizuka1, K. Noguchi1, A. Kita, M. Odaka1, K. Miki and M. Yohda, J. Mol. Biol. 376, 1130 (2008). http://dx.doi.org/10.1016/j.jmb.2007.12.010

[19] T. Zako, R. Iizuka, M. Okochi, T. Nomura, T. Ueno and H. Tadakuma, FEBS Lett. 579, 3718 (2005). http://dx.doi.org/10.1016/j.febslet.2005.05.061

[20] R. Siegert, M. R. Leroux, C. Scheufler, F. U. Hartl and I. Moarefi, Cell 103, 621 (2000). http://dx.doi.org/10.1016/S0092-8674(00)00165-3

[21] R. Iizuka, Y. Sugano, N. Ide, A. Ohtaki, T. Yoshida, S. Fujiwara, T. Imanaka and M. Yohda, J. Mol. Biol. 377, 972 (2008). http://dx.doi.org/10.1016/j.jmb.2008.01.070

[22] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, Nucleic Acids Res. 28, 235 (2000). http://dx.doi.org/10.1093/nar/28.1.235

[23] H. Li, A. D. Robertson and J. H. Jensen, Proteins 61, 704 (2005). http://dx.doi.org/10.1002/prot.20660