Iranian Journal of Analytical Chemistry
Volume 8, Issue 1, March 2021 (50-55) Origonal Research Article

مجله ایرانی شیمی تجزیه
دوره۸، شماره ۱، فروردین ۱۴۰۰ (۵۵-۵۰)

Molecular Docking and Molecular Dynamics Studies of Ectoine Drug and Polyamidoamine/Ectoine Conjugates With the 6twk Protein

Nosrat Madadi Mahani
Department of Chemistry, Payame Noor University, 19395-4697 Tehran, Iran
Received: 16 January 2021 **Accepted:** 6 March 2021
DOI: 10.30473/ijac.2021.59403.1193

Abstract
The interactions of 6twk protein with Ectoine drug, Polyamidoamine (PAMAM) /Ectoine and histidine modified PAMAM/Ectoine were investigated using molecular docking and molecular dynamics simulation. Based on the results of molecular docking increasing of binding energy and the decreasing of inhibition constant of the compounds, increase their inhibitory activity. Protein stability in complex with these ligands was investigated using molecular dynamics simulation approach. Results molecular dynamics simulation displayed that histidine modified PAMAM/Ectoine with the lowest mean square displacement (MSD) is the better suitable to deliver the ectoine drug. This causes the most controlled/diffusion of ectoine drug molecule. So, histidine modified PAMAM/Ectoine conjugate can be introduced for further investigations on interaction of ectoine drug and 6twk protein.

Keywords: Molecular Docking; Inhibitory Activity; Molecular Dynamics; PAMAM/Ectoine Conjugate.

1. INTRODUCTION

Different types of nanocarriers including polymers, liposomes, poly-ion complex micelles, cell-penetrating peptides and dendrimers have been utilized in anticancer drug delivery systems [1-3]. Among these, dendrimers are polymers with highly branched architectures of nanometer dimensions are showed the potential applications in bio-imaging, drug delivery, and gene transfer [4, 5]. Dendrimer is a well-known polymer, which is capable of binding with drugs and proteins through covalent, noncovalent, hydrophobic and hydrophilic interactions [6]. As compared to other polymers, dendrimers showed less toxicity in animals and now they are under human trials for commercial usage in cancer treatment [7, 8].

Polyamidoamines dendrimers with greatly special and hierarchical three-dimensional architecture, are one of the dendrimers which are highly utilized as proper carriers to delivery drugs and tissue engineering and regenerative medicine [9, 10]. Wu et al. were reported that the histidine modified PAMAM dendritic can be applied as efficient chemotherapeutic drug vehicles in cancer treatment [11]. The bonding of drug-dendrimer are so significant in pharmaceutical industry, the biomedical sciences and pharmaceutical industry. Ectoine drug is considered as one natural metabolite that force the apoptosis in the cells of lung cancer [12]. It could be applied as a medicine for the treatment of lung cancer after more optimization of formulations or as auxiliary drug. Since it has no toxic impact on other cells [13, 14].

Molecular docking may be a simulation approach for planning drug which can be predict the compliance of a receptor-ligand complex. The receptor is regularly a protein or a nucleic acid molecule (DNA or RNA) and the ligand could be a small particle or drug [15]. Besides, molecular docking is one of main instrument for virtual screening strategies. Molecular docking analysis of PAMMA–5FU with the oncoproteins (E6 and E7) has been studied by Rengaraj et al. that displayed PAMAM/5-fluorouracil drug conjugate had a higher affinity for the oncoprotein than for 5-FU [16].

Molecular dynamics simulations are broadly accomplished to understand physical phenomena occurring in various membranes at the molecular level [17-20]. MD simulation is very helpful in analyzing the conformational stability and dynamics of the protein and protein-ligand complexes at different nanosecond time intervals, fluctuations, and their deviations from the reference structure that will be discussed in the study. The interactions of compounds ectoine, PAMAM-ectoine and histidine modified PAMAM- ectoine with 6twk protein were studied by molecular docking method. The molecular dynamics (MD) simulation was applied for stability of protein complex with compounds.

2. EXPERIMENTAL

Docking calculations between ectoine, PAMAM@ectoine and Histidine modified PAMAM@ectoine as ligand and 6twk protein have been performed using Auto Dock 4.2[21].

*Corresponding Author: nmmadady@gmail.com

Crystal structure of 6twk protein has been gained from Protein Data Bank (www.rcsb.org). This protein has two chains A and B that A was omitted. Then, molecular docking was accomplished using the lamarckian genetic algorithm. To identify the binding sites in 6twk protein, blind docking has been done, By the grid size set to 60, 60, and 60 Angstrom along with X-, Y- and Z-axes with 9.526, 22.699 and -23.362^{0}A grid space. The conformation with the lowest binding free energy and low inhibition constants was employed for better analysis.

In Molecular dynamics simulations, the motion and distribution of particles and the dynamical behavior of the system are calculated based on Newton's motion equations and statistical mechanics. This method is used to predict the specific behavior of macromolecules in different conditions. In this method, the interparticle forces and the potential energy of the system are defined by molecular mechanics force fields. In the minimization and dynamics steps of all molecules, the condensed-phase optimized molecular potential (COMPASS) force field (FF) was employed[22].which is the first FF validated and parameterized by means of condensed-phase criteria and ab initio and empirical data. The COMPASS force field is frequently used to investigate the drug delivery efficacy of various drug delivery systems. MD simulations were done on efficiency of pharmacokinetic of PAMAM-dendrimer-furosemide delivery systems [23], self-assembled paclitaxel structures [24] and Loading of doxorubicin onto the functionalized graphene surface by employing the COMPASS force field [25]. Materials Studio software was used to perform the MD simulation runs [26].

The minimization of energy of amorphous cell and balancing stages (NVT and NVE), were done using the Forcite module. The Mean square displacement (MSD) and the radius of gyration (Rg) as the as a function of time were analyzed. These parameters are used investigation of sustainability of the structure of protein.

3. RESULTS AND DISCUSSION

A molecular docking is used for prediction the structure of the receptor-ligand complex and investigation of the interactions of ligands and receptor in the process of discovery of new pharmaceutical compounds. Crystallographic structure of 6twk (B) protein and structure of Ectoine drug were displayed in Fig. 1.

Due to Fig. 2, docking of medicinal Ectoine drug with protein 6twk (B) were detailed with Discovery Studio visualizer 4.1 (2014) software. The docking studies showed that ectoine interacted at two sites of the 6twk (B), through Arg 322 (H) and Glu 255 (O) of the 6twk (B) via O and N atoms with bond distances of 2.97 and 2.88 A, respectively. The ligand forms stable complex and has good binding energy values (-4.58 kcal/mol). PAMAM/Ectoine and histidine modified PAMAM/Ectoine were docked with 6twk (B) and the observed binding energy were -5.63 and -6.57 kcal/mol, respectively. The docking studies showed that PAMAM/Ectoine interacted at three sites of the 6twk (B), through Glu 255, Asp 338, and Pro 337.While histidine modified PAMAM/Ectoine interacted at Tryp 339 site of the 6twk (B). PAMAM/Ectoine and histidine modified PAMAM/Ectoine displayed the minimum of inhibition constant (Ki) which have more activity (22.63 and 20.38 μM) than the pristine Ectoine (41.25μM).

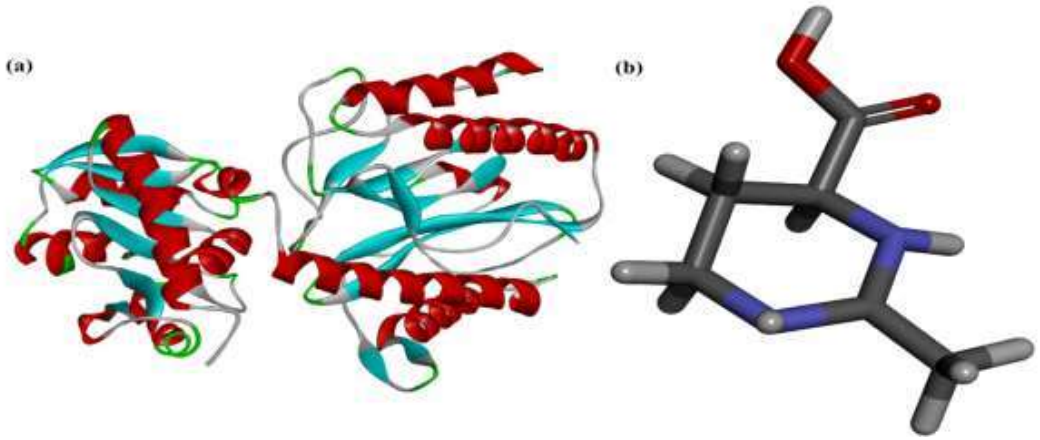

Fig. 1. (a) Crystallographic structure of 6twk protein (B), (b) Ectoine drug .

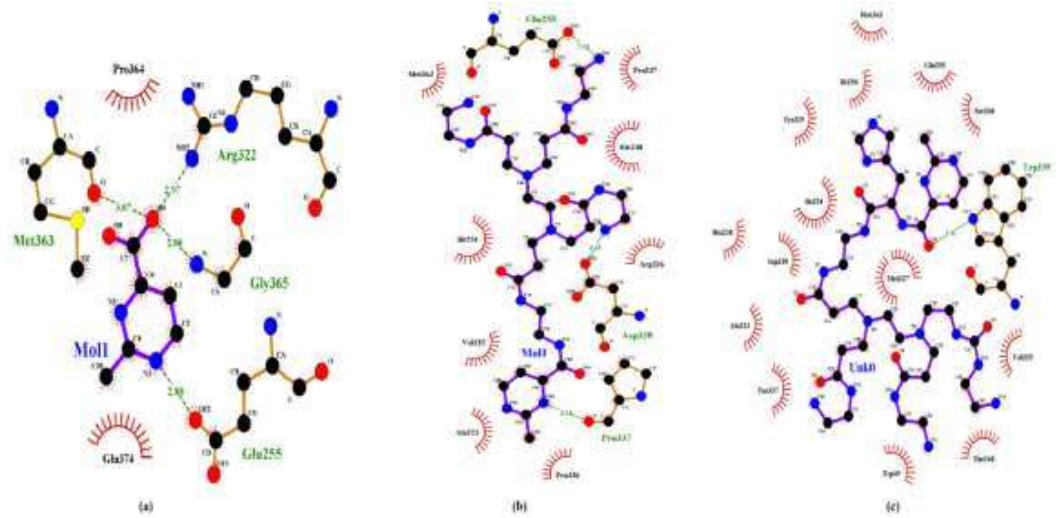

Fig. 2. Docking of Ectoine (a), PAMAM/Ectoine and histidine modified PAMAM/Ectoine (c) with 6twk protein (B)

A common metric used to evaluate distance between the predicted pose and the native pose, given a superposition of their protein receptor structures, is the root mean square deviation (RMSD) between their respective atoms (Eq. 1):

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N} d_i^2} \qquad (1)$$

Where N is the number of atoms in the ligand, and d_i is the Euclidean distance between the i_{th} pair of corresponding atoms [28]. The RMSD curve of 6twk protein over PAMAM/Ectoine and histidine modified PAMAM/Ectoine conjugates are shown in Fig. 3. To investigation the possible orientation of these molecules during the interaction, RMSD values were calculated for 10 samples with 1.0 picosecond equilibration and a trajectory of 100 picoseconds. The RMSD values showed more fluctuation because of the formation of a complex between the protein and the dendrimer in solution. The PAMAM/Ectoine and histidine modified PAMAM/Ectoine posse an equal number of binding orientations with protein and they illustrated more possible orientations.

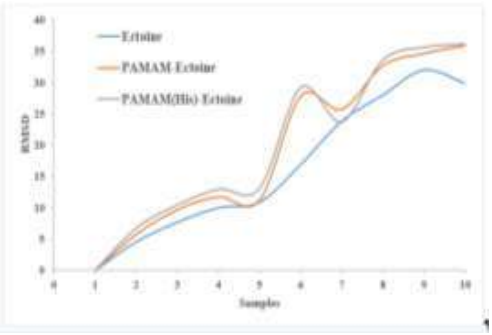

Fig. 3. RMSD plot of 6twk protein over Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine conjugates.

The diffusion ability of the ligands Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine conjugates inside the 6twk protein is estimated from the mean square displacement (MSD) plots, and the diffusion coefficient (D) values can be computed from the slopes of these plots. The MSD diagrams for the ligands Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine conjugates molecule in the 6twk protein drug delivery systems are presented in Fig. 4. The almost linear lines in the MSD (mean square displacement) curves confirm the continuous ligands diffusion within the systems during the MD simulations. Also, it is apparent that the Ectoine demonstrates the highest MSD for diffusion while histidine modified PAMAM/Ectoine conjugate displays the smallest MSD. Therefore, in order to perform an effective drug delivery, a system with the lowest MSD is essential.

The radius of gyration (Rg) represents the size of ligand& protein complex, defined as follows:

$$< R_g^2 >= \frac{1}{M} < \sum_{i=1}^{N} m_i |r_i - R|^2 > \qquad (2)$$

Where M is the mass of the whole complex, m_i the mass of an atom i, r_i the position of an atom i, and R the center of mass of the complex [29]. < > mentions to average value in a NVT ensemble (T = 298 K).

Changes in protein size in the presence of ligands of Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine during simulations time are shown in the Fig. 5. As shown in the graph, the radius of gyration increases to 68 ns in the presence of all ligands. However, after that time, a decreasing was observed in the radius. But, after 89 ns, the increasing rate is reduced and the radius of gyration shows a lower fluctuation until 20 ns. The radius of gyration describes the

dimension of a protein chain. Larger Rg is indicative of a larger distance between the protein chain and its mass center. Also, the Ectoine shows the greatest Rg and histidine modified PAMAM/Ectoine displays the smallest R_g.

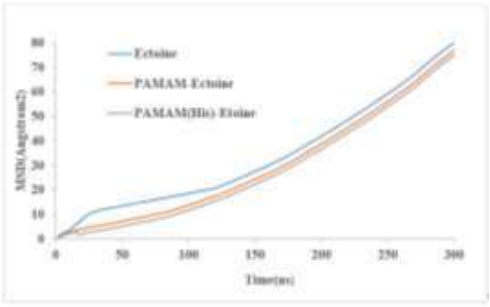

Fig. 4. The MSD diagrams for the diffusion of Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine conjugates inside the 6twk protein.

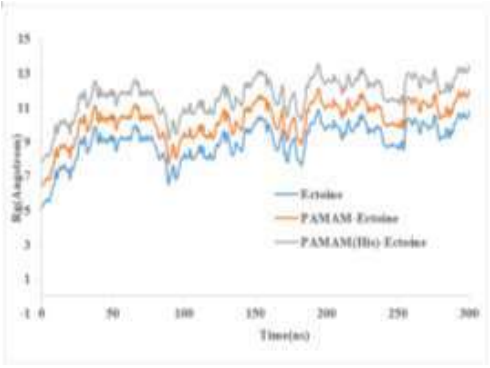

Fig. 5. The time evolutions of the gyration (Rg) of 6twk protein as a function of time in complex with ligand Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine.

4. CONCLUSIONS

Hydrogen and hydrophobic interactions were investigated by molecular docking. The docking studies showed that binding energy of histidine modified PAMAM/Ectoine with 6twk (B) was -6.57 kcal/mol. Also Histidine modified PAMAM/Ectoine displayed the minimum of inhibition constant (Ki) which have more activity. The MD simulations were accomplished on the ligands of Ectoine, PAMAM/Ectoine and histidine modified PAMAM/Ectoine having 6twk protein. The almost linear lines in the MSD curves confirm the continuous ligands diffusion within the systems during the MD simulations. For performing an effective drug delivery, histidine modified PAMAM/Ectoine system with the lowest MSD is suitable. Also, histidine modified PAMAM/Ectoine displays the smallest R_g that indicative a smaller distance between the protein chain and its mass center. The results can be useful for design and development of drug delivery conjugates.

Acknowledgement

The author is grateful to the Payame Noor University for encouragements.

REFERENCES

[1] L. Nair, S. Jagadeeshan, S.A. Nair and G.V. Kumar, Biological evaluation of 5-fluorouracil nanoparticles for cancer chemotherapy and its dependence on the carrier PLGA, *Int. J. Nanomedicine* 6 (2011) 1685-1697.

[2] A. Rengaraj, P. Puthiaraj, Y. Haldorai, N.S. Heo, S.K. Hwang, Y.K. Han, S. Kwon, W.S. Ahn and Y.S. Huh, Porous Covalent Triazine Polymer as a Potential Nanocargo for Cancer Therapy and Imaging, *ACS Appl. Mater. Interfaces* 8 (2016) 8947–8955.

[3] K. Ariga, K. Kawakami, M. Ebara, Y. Kotsuchibashi, Q. Ji and J.P. Hill, Bioinspired nanoarchitectonics as emerging drug delivery systems, *New J. Chem.* 38 (2014) 5149–5163.

[4] U. Boas and P.M. Heegaard, Dendrimers in drug research, *Chem. Soc. Rev.* 33 (2004) 43–63.

[5] C. Dufes, I.F. Uchegbu and A.G. Schatzlein, Dendrimers in gene delivery, *Adv. Drug Delivery Rev.* 57 (2005) 2177–2202.

[6] Y. Cheng, Z. Xu, M. Ma and T. Xu, Dendrimers as drug carriers: applications in different routes of drug administration, *J. Pharm. Sci.* 97 (2008) 123–143.

[7] S. Sadekar and H. Ghandehari, Transepithelial transport and toxicity of PAMAM dendrimers: implications for oral drug delivery, *Adv. Drug Delivery Rev.* 64 (2012) 571–588.

[8] C. Pan, C. Kumar, S. Bohl, U. Klingmueller and M. Mann, Comparative proteomic phenotyping of cell lines and primary cells to assess preservation of cell type-specific functions, *Mol. Cell. Proteomics* 8 (2009) 443–450.

[9] X. Zhang, Y. Zeng, T. Yu, J. Chen, G. Yang and Y. Li, Tetrathiafulvalene terminal-decorated PAMAM dendrimers for triggered release synergistically stimulated by redox and CB, *Langmuir* 30 (2014) 718–726.

[10] F. Aulenta, W. Hayes and S. Rannard, Dendrimers: a new class of nanoscopic containers and delivery devices, *Eur. Pol. J.* 39 (2003) 1741-1771.

[11] S.Y. Wu, H.Y. Chou, H.C. Tsai, R. Anbazhagan, C.H. Yuh, J. M.Yang and Y.H. Chang, Amino acid-modified PAMAM

dendritic nanocarriers as effective chemotherapeutic d rug vehicles in cancer treatment: a study using zebrafish as a cancer model, *RSC Adv.* 10 (2020) 20682- 20690.

[12] M. Sheikhpour, A. Sadeghi, F. Yazdian, A. Movafagh and A. Mansoori, Anticancer and apoptotic effects of ectoine and hydroxyectoine on non-small cell lung cancer cells: An in-vitro investigation, *Multidiscipl. Cancer Invest.* 3 (2019) 14-20.

[13] J.M. Pastor, M. Salvador, M. Argandona, V. Bernal, M. Reina-Bueno, L.N. Csonka, J.L. Iborra, C. Vargas, J.J. Nieto and M. Canovas, Ectoines in cell stress protection: Uses and biotechnological production, *Biotechnol. Adv.* 28 (2010**)** 782–801.

[14] M.B. Hahn, S. Meyer, M.A. Schroter, H. J. Kunte, T. Solomun and H. Sturm, DNA protection by ectoine from ionizing radiation Molecular mechanisms. *Phys. Chem. Chem. Phys.* 19 (2017) 25717–25722.

[15] R. Dias and W. Filgueira de Azevedo Jr, Molecular docking algorithms, *Curr. Drug Targets* 9 (2008) 1040-1047.

[16] A. Rengaraj, B. Subbiah, Y. Haldorai, D. Yesudhas, H.J. Yun, S. Kwon, S. Choi, Y.K. Han, E.S. Kim, H. Shenpagam and Y.S. Huh, PAMAM/5-fluorouracil drug conjugate for targeting E6 and E7 oncoproteins in cervical cancer: a combined experimental/in silico approach, *RSC Adv.* 7(2017) 5046-5054.

[17] Z. Shariatinia, A. Mazloom Jalali and F. Afshar Taromi, Molecular dynamics simulations on desulfurization of n-octane/thiophene mixture using silica filled polydimethylsiloxane nanocomposite membranes, 24 (2016) *Model Simul. Mater. Sci. Eng.* 035002- 035011.

[18] A. Mazloom Jalali, Z. Shariatinia and F. Afshar Taromi, Desulfurization efficiency of polydimethylsiloxane/silica nanoparticle nanocomposite membranes: MD simulations, *Comput. Mater Sci.* 139 (2017) 115–124.

[19] S. Salar, F. Mehrnejad, R.H. Sajedi and J. Mohammadnejad Arough, Chitosan nanoparticles-trypsin interactions: biophysicochemical and molecular dynamics simulation studies, *Int. J. Biol. Macromol.* 103 (2017) 902–909.

[20] M. Yahyaei, F. Mehrnejad, H. Naderi-manesh and A.H. Rezayan, Follicle-stimulating hormone encapsulation in the cholesterolmodified chitosan nanoparticles via molecular dynamics simulations and binding free energy calculations, *Eur. J. Pharm. Sci.* 107(2017) 126–137.

[21] G. Morris and R. Huey, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.* 30 (2009) 2785-2791.

[22] H. Sun, COMPASS: an ab initio force-field optimized for condensed-phase applications overview with details on alkane and benzene compounds, *J. Phys. Chem. B* 102(1998) 7338–7364.

[23] D.P. Otto and M.M. de Villiers, All-atomistic molecular dynamics (AA-MD) studies and pharmacokinetic performance of PAMAM-dendrimer-furosemide delivery systems, *Int. J. Pharm.* 547(2018) 545–555.

[24] X.D. Guo, J.P.K. Tan, S.H. Kim, L.J. Zhang, Y. Zhang, J.L. Hedrick, Y.Y. Yang and Y. Qian, Computational studies on self-assembled paclitaxel structures: templates for hierarchical block copolymer assemblies and sustained drug release, *Biomaterials* 30 (2009) 6556–6563.

[25] M.M. Mirhosseini, M. Rahmati, S.S. Zargarian and R. Khordad, Molecular dynamics simulation of functionalized graphene surface for high efficient loading of doxorubicin, *J. Mol. Struct.* 1141(2017) 441–450.

[26] Accelrys Software Inc., San Diego, (2017).

[27] K. Sargsyan, C. Grauffel and C. Lim, How molecular size impacts RMSD applications in molecular dynamics simulations, *J. Chem. Theory Comput.* 13(2017) 1518−1524.

[28] Y. Liu, V.S. Bryantsev and M.S. Diallo, et al., PAMAM dendrimers undergo pH responsive conformational changes without swelling, *J. Am. Chem. Soc.* 131 (2009) 2798–2799.

مطالعات داکینگ مولکولی و دینامیک مولکولی داروی اکتوئین و مزدوج اکتوئین- دندریمر پلی امیدوامین با پروتئین6twk

نصرت مددی ماهانی

بخش شیمی، دانشگاه پیام نور، صندوق پستی ۳۶۹۷-۱۹۳۹۵ ، تهران، ایران

تاریخ دریافت: ۲۹ دیماه ۱۳۹۹ تاریخ پذیرش: ۱۸ اسفند ۱۳۹۹

چکیده

برهمکنش‌های پروتئین 6twk با داروی اکتوئین، مزدوج اکتوئین- دندریمر پلی‌امیدوامین و مزدوج اکتوئین- دندریمر پلی امیدوامین اصلاح‌شده با هیستیدین با استفاده از داکینگ مولکولی شبیه‌سازی دینامیک مولکولی بررسی گردید. بر اساس نتایج داکینگ مولکولی، افزایش انرژی پیوندی و کاهش ثابت بازداری ترکیبات، فعالیت بازداری آنها افزایش می یابد. پایداری پروتئین در کمپلکس با این لیگاندها با استفاده از شبیه‌سازی دینامیک مولکولی مطالعه گردید. نتایج شبیه‌سازی دینامیک مولکولی نشان داد که کمپلکس پروتئین با دندریمر پلیمری اصلاح شده با هیستیدین- اکتوئین، کمترین مقدار میانگین مربع جابجایی، مناسب‌ترین لیگاند برای پروتئین و جابجایی دارو می‌باشد و این باعث می‌شود که نفوذ و کنترل دارو بهتر انجام می‌شود. همچنین این سیستم می‌تواند برای بررسی بیشتر بر روی برهم کنش‌های دارو اکتوئین و پروتئین معرفی گردد.

واژه‌های کلیدی

داکینگ مولکولی؛ دینامیک مولکولی؛ فعالیت بازدارندگی؛ مزدوج اکتوئین- دندریمر پلی امیدوامین.