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Research Article

Free Energy Calculation for NavAb Channel Using the Crooks Fluctuation Method

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Abstract

Membrane proteins such as ion channels, transporters and receptors are among the biologically important proteins. Because membrane proteins allow cells to communicate with their environment. Therefore, membrane proteins are targets for almost all drugs. It is also of scientific interest that membrane proteins play an important role in the control of the life process. Recent developments in experimental and computational sciences have further increased the interest in membrane proteins. The milestone development in this area is the obtaining of the crystal structure of membrane proteins. The crystal structure of the voltage-gated bacterial Nav channel was obtained in 2011 by Payandeh et al. Voltage-gated sodium channels are very important as they initiate the action potential. In this study, the Crooks fluctuation method, which was developed for the calculation of free energy in unbalanced systems, was applied for voltage-gated sodium channels, which is a complex biological system. The results were compared with other calculation methods in the literature.

Keywords: Crooks fluctuation method, Ion channels, Sodium channel, Molecular modelling

1. INTRODUCTION

Recently one of the greatest discoveries of ultramodern science is the application of physical thought to life [1,2]. As is known, the laws of thermodynamics are applied to biophysical problems [3]. The laws of thermodynamics come into play when a physical description of a living system is possible [4]. Until the early 1990s, researchers did not know how small systems do work and heat exchange with their environment, and the experimental methods needed to understand these properties [5]. The development of ultramodern techniques has been thanks to microscopic manipulations and thus new experiments have been developed. There have been fluctuation theorems developed and experimentally tested by theorists over the past two to three decades [5,6]. Advanced experiments with energy fluctuations of small systems are now being done. The principles that provide solutions to both energy changes and statistical deviations of these systems have been formulated and started to shed light on the unique properties of microscopic systems. [5,7,8].

As a result, physicists have developed new experimental and theoretical tools that can serve as the basis for a non-equilibrium thermodynamic theory of small systems. Thanks to these tools, they have gained knowledge about small systems and continue to gain. Simulations with advanced computer systems are useful techniques for understanding molecular structures and gaining new and deeper insights into microscopic interactions. Microscopic properties of systems can be predicted by combining simulations with macroscopic experimental observations. Today, computer simulations have become an important part of the mathematical modeling of natural systems [5,9].

Molecular Modelling (MM) methods are used to study biological systems at the atomic scale. MM methods cover quantum mechanics and classical mechanics. Minimization processes, dynamic simulations, conformational analysis and other computer-based methods are used to understand and predict the structural and functional properties of the molecular system using MM [10]. Here, the first step is the selection of a model for intramolecular and intermolecular interactions in the system. These selected models allow the arrangement of atoms and molecules in the system, the calculation of energy, and how to determine the energy of the system, which differs with the positions of its atoms. The second step is to perform energy minimization to find the most stable structure possible. Finally, the results of the calculation are compared with the experimental data. When the simulation results are compared with the experimental results, certain features of the system can be analysed if consistent results are observed. In summary, there is a close

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connection between theory and experiment: computational models evolve as empirical data becomes available; As calculations are concluded, new experiments are organized, and biological theories are developed.

2. MATERIAL AND METHODS

2.1 Free Energy

Free energy is the most important data that plays a role in understanding dynamic processes. Molecular Dynamics (MD) simulations give veritable useful information about the dynamic behavior of the system at the atomic level [9,11]. Simulation trajectory data analysis; It can explain many structural features such as bond, angle, and torsion angles, as well as hydrogen bonds between atoms. In order to fully understand the system, the free energy behavior of chemical processes should be studied. MD simulations can provide useful insights into the membrane-protein mechanism and ion transition dynamics, and most of the experimental characteristics can be inferred from the free energy information of the system [12,13]. Free energy calculations are used to investigate the energy values in ion and ligand transport events from ion channels or transporters. In addition, free energy calculations are made in processes such as protein folding, protein-protein interactions and protein-ligand binding. The calculated free energy can define the state of the membrane-protein system, or the free energy difference between any two states in an ion channel can be calculated [14–16].

Although there are various approaches to obtain free energy in various membrane-protein systems, accurate estimation of free energy in some large biological structures is quite difficult. By knowing the free energy difference associated with the state change of the system, it is possible to establish stable states. It is also possible to understand the thermodynamic properties of steady states, the kinetics of transitions between states, and how stable states are affected by external conditions. Free energy calculations play a key role in establishing the structure-function linkage, as it provides perturbation and structural analysis of the simulated systems at the atomic level [13,16].

There are path-dependent and path-independent methods for calculating free energy in molecular simulations. Path dependent methods are used to calculate the absolute binding energy. Path-independent methods are used to calculate the relative binding energy. With absolute free energy calculation, this method can be used, but it is quite difficult, especially for charged ions [16].

2.2 Crooks Fluctuation Theorem

Gavin Crooks produced an important fluctuation theorem obtained earlier by Christopher Jarzynski. In Jarzynski's setup, as well as in Crooks's generalized fluctuation theory, the system is initially in thermal equilibrium, but is then moved out of equilibrium by the action of an external agent [5,17].

When work is done in a non-equilibrium system, the average work $\langle W \rangle$ done is given by the free energy difference between (ΔG) the initial and final states, with an external variable parameter λ of the system [18].

$$\langle W \rangle \geq \Delta \mathrm{G} \tag{1}$$

The non-equilibrium work theorem or Jarzynski equation is given as a special case of the mean of non-equilibrium work, as follows [19].

$$\langle e^{-\beta W} \rangle = e^{-\beta \Delta G} \tag{2}$$

Here $\beta = 1/k_{\beta}T$. The Crooks fluctuation theorem [17] is about the relationship between non-equilibrium probability distributions of work done on and by the system when λ changes in forward (F) and backward (B) directions under predetermined conditions.

$$\frac{P_F(W)}{P_B(-W)} = e^{-\beta(\Delta G - W)} \tag{3}$$

Here $P_F(W)$ and $P_B(W)$ represent the work-probability distributions along the forward and backward processes, respectively. In the simplest case, λ is increased by a constant rate for the forward process and decreased by the same constant rate for the backward process.

The crooks fluctuation theorem is a more general equation and the Jarzynski equation is derived from this distribution theorem. There are two interesting points about this theorem [18]. Firstly, using non-equilibrium statistical physics to verify these exact equations, secondly, it is to organize non-equilibrium experiments and simulations on biological molecules to observe free energy differences.

An important feature of the fluctuation theorem is related to the choice of the increment value of λ. This is true for the protocol that connects $\lambda = 0$ and $\lambda = 1$ over an arbitrary period of time. However, work intensities are functions of protocols, and their formats depend on our choice. But in each case the forward and backward work functions are linked by the above equation. In particular, the work functions are always different from each other, they intersect only when $W = \Delta F$, as shown in Figure 1.

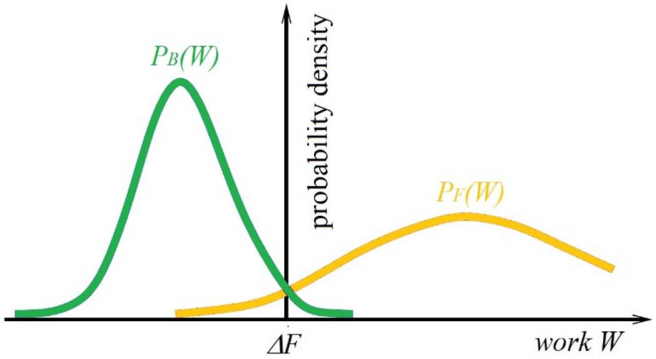


Figure 1. The forward and backward work densities are shown $P_F(W)$ and $P_B(W)$, respectively, and the work densities intersect at the equilibrium free energy difference ΔF

As a result of the distribution theorem consistent with the second law of thermodynamics, the mean backward work $P_B(W)$ is smaller than ΔF, while the mean forward work $P_F(W)$ is greater than ΔF.

2.3 NavAb Ion Channels

Some of the proteins in the cell membrane form channels across the membrane and are called ion channels. Ion channels help living organisms to perform various tasks from sensing movement by generating action potentials in nerves, muscles, and synapses [20]. To achieve this goal, the channel needs to deliver thousands of ions when opened over a period of a few milliseconds. The ion channels are very narrow (with a radius of several Å) and therefore ions usually move in a single line [21–25]. Another outstanding feature that distinguishes biological ion channels from being just a hole in the membrane wall is their selectivity. In other words, ion channels are designed to transmit certain types of ions.

In this study, the NavAb channel was studied as the ion channel. NavAb channels play a central role in physiology. They also regulate electrical signals and information in the nervous system. Nav channels initiate action potentials. Hereditary epilepsy, migraine, periodic paralysis, cardiac arrhythmia and chronic pain syndromes occur with the deterioration of the Nav channels. These channels are; They are molecular targets of drugs used in local anesthesia, genetic therapy, and sporadic Nav channelopathies in the brain, skeletal muscle, and heart [26–29]. The rapid activation, sodium ion selectivity of Nav channels, and drug sensitivity are unique among voltage-gated ion channels.

The NavAb structure found by Payandeh et al provides a basis for the selectivity and high conductivity of Nav channels [1]. As shown in Figure 2a, the channel portion of the NavAb construct consists of a funnel-like inlet, a selective filter, a central cavity, and an intracellular activation gate.

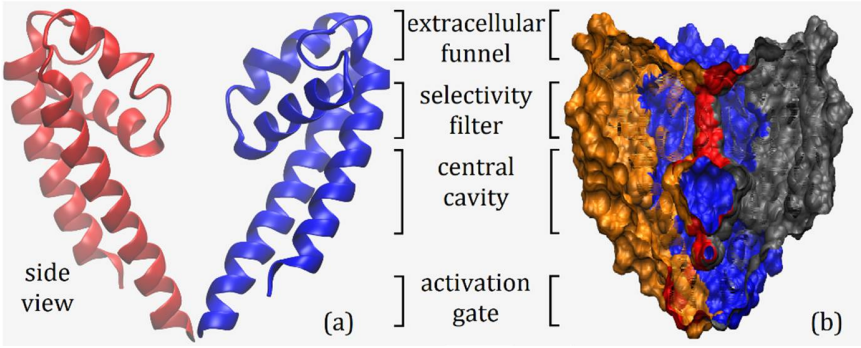


Figure 2. (a) View of the channel portion of the NavAb construct, (b) View of the hydrophobicity of the channel portion of the NavAb structure

The large central cavity in the NavAb structure is wide enough to easily accommodate a Na^+ ion and its hydration shell, and it has a hydrophobic surface on the structure that allows ions to diffuse rapidly (Figure 2b). The ion conduction pathway in the NavAb structure is strongly electronegative and the channel narrows on the extracellular side of the selective filter. The channel filter structure consists of amino acids W179, S178, E177, L176 and T175. The residues between T175 and W179 form a tight structure, the T175 and L176 backbone carbonyls play a role in the conduction of ions.

3. RESULTS AND DISCUSSION

3.1 Simulation Details

The crystal structure of the protein was downloaded from PDB (3RVY) [1]. The tetramer structure of the NavAb channel was provided and removed from the structure to reduce the voltage-sensing domain (1-113 residues) in the crystal structure and increase the computational efficiency. With the help of the VMD program [30], palmitoyl-oleoylphosphatidylcholine (POPC) lipids were selected and a bilayer consisting of 113 lipid chains (15142 atoms) in the -xy plane of $85x85$ Å^2 dimension was obtained, and the protein was embedded in this structure. An ion concentration of 0.15 M was added (42 sodium, 26 chlorine atoms) to neutralize the system and provide the physiological value. In order to make the structure embedded in the membrane similar to the physiological environment, a membrane protein system was established by adding a total of 9310 water molecules (27930 atoms) to the system. In total, there are 49248 atoms in the system and the system size is $85x85x80$ Å^3. The view of the system is given in Figure 3.

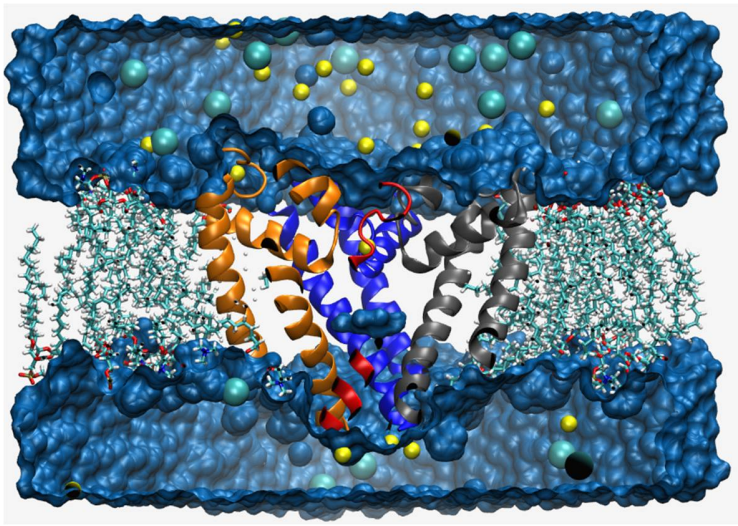


Figure 3. View of the NavAb system

All MD simulations were performed using version 2.8 of the NAMD [31] program and the Charmm force field was used [32]. Periodic boundary conditions were applied for all directions. Long-range electrostatic interactions were made in the NPT community using the PME algorithm. The Langevin Piston method was used to provide 1 atm pressure with 50 fs piston distortion and 100 fs piston period. For Van der Walls interactions, the cut-off distance was kept at 12 Å and the switching distance at 10 Å. During the simulation, the time step was taken as 2 fs and the simulation data were recorded at every 1 ps intervals.

3.2 Balancing the Protein System

In this process, since the distance between some atoms that make up the system is likely to be less than the van der Waals distances, the system was minimized in 1000 MD steps as a first step. During this time, the system temperature was brought to 300° K and the pressure to 1 atm. From our experience, minimizing so many steps have eliminated the so-called bad interaction distances. After the system is established, the system must first come to equilibrium. During the process of balancing the system, harmonic forces (constraints) were applied to the backbone and other heavy atoms of the protein structure. The aim here is to adapt the protein together with lipid and water atoms and to provide NPT simulation conditions in the system. The harmonic forces on the protein were reduced at certain time intervals and the simulation was continued until the force constant k was 0.1 kcal/mol/Å² [16].

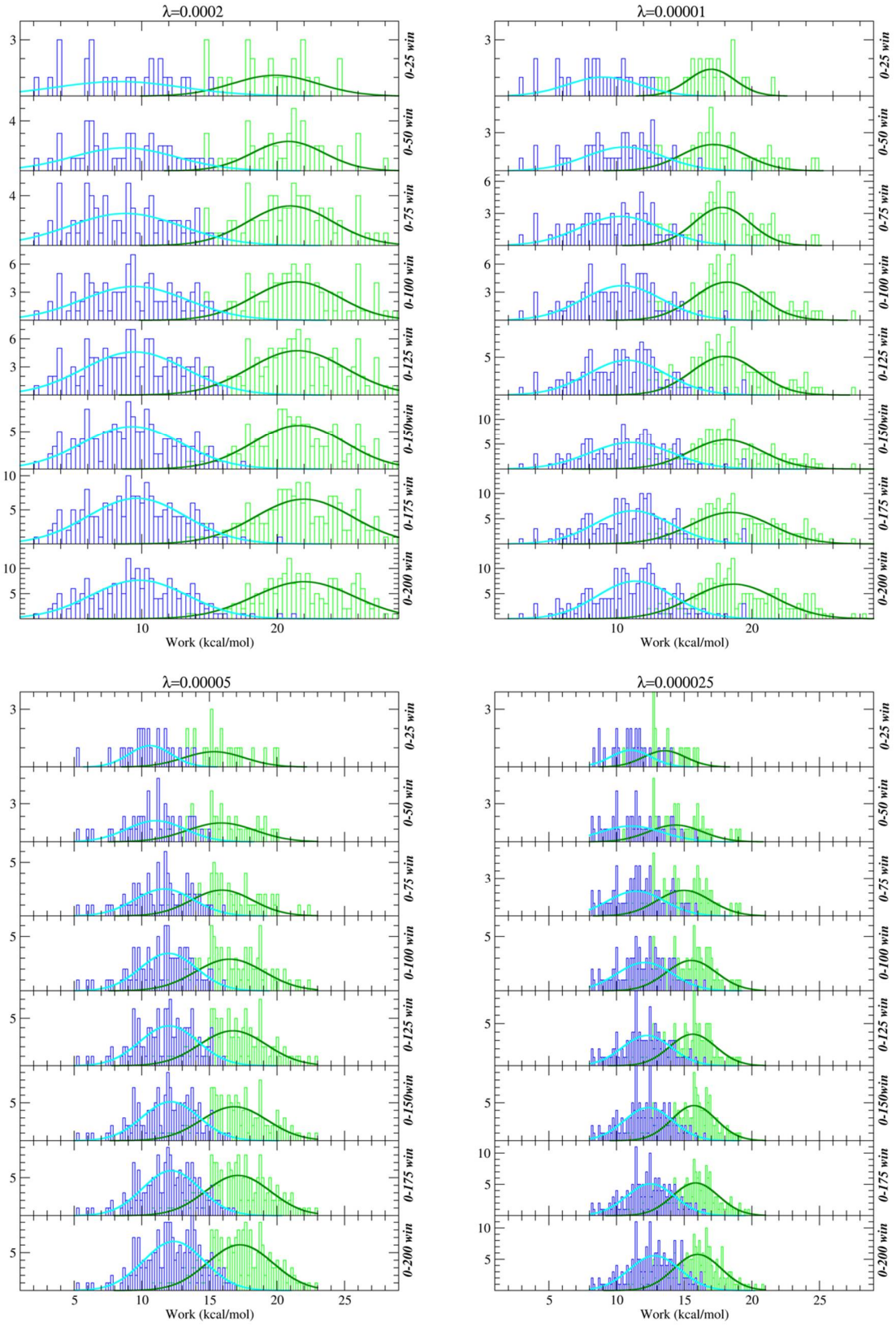
During the equilibrium process of the system, it was observed that the density of the system was approximately 1.0 kg/l and the system came to equilibrium. During the equilibrium process, it is also seen that the backbone structure of each protein chain has a maximum RMSD value of 0.4 Å. After the system was brought to equilibrium, a very small harmonic constraint (0.1 kcal/mol) was applied to the backbone atoms of the ion channel and MD was performed for 200 ns. During this period, the RMSD value of the protein backbone structure is seen as maximum 0.5 Å.

3.3 Crooks Calculation

After the system was brought to equilibrium, energy calculation studies were started using the Crooks Theorem. As stated in the literature, our aim here is to calculate energies with a large number of short-term simulations. It is to find a net energy with the point where the curves intersect by fitting the histograms of the energies in the forward and backward state to the gauss curve. Initially, 200 batch jobs of 100 ps were run. A total of 200 samples were obtained at the end of a 20 ns simulation. For 7 different λ values, free energy calculations were made in this example works in forward orientation and then in backward orientation. As stated above in the Crooks fluctuation theorem, the protocol connection between $\lambda = 0$ and $\lambda = 1$ is very important. Forward and backward operations were performed by keeping the λ values determined between $\lambda = 0$ and $\lambda = 1$ equal. This process is similar in FEP theory, but for each λ value, while 20000 steps are run in the FEP calculation, we have run 1 step of work in this method (This will give us at least 100 times the CPU time gain). However, we keep the number of samples too high because we are conducting a method development tests.

Table 1. Value of λ, Number of Steps, Number of sample and CPU time

Value of $\boldsymbol{\lambda}$	Number of Steps	Number of sample (fw/bw)	CPU Time for 1 sample (second)
0,0002000	5000	200/200	4000
0,0001000	10000	200/200	8000
0,0000500	20000	200/200	16000
0,0000250	40000	200/200	32000
0,0000125	80000	200/200	64000
0,0000100	100000	200/200	80000
0,0000050	200000	200/200	160000


λ=0.0002
λ=0.00001
λ=0.00005
λ=0.000025
0-25 win
0-50 win
0-75 win
0-100 win
0-125 win
0-150win
0-175 win
0-200 win
Work (kcal/mol)

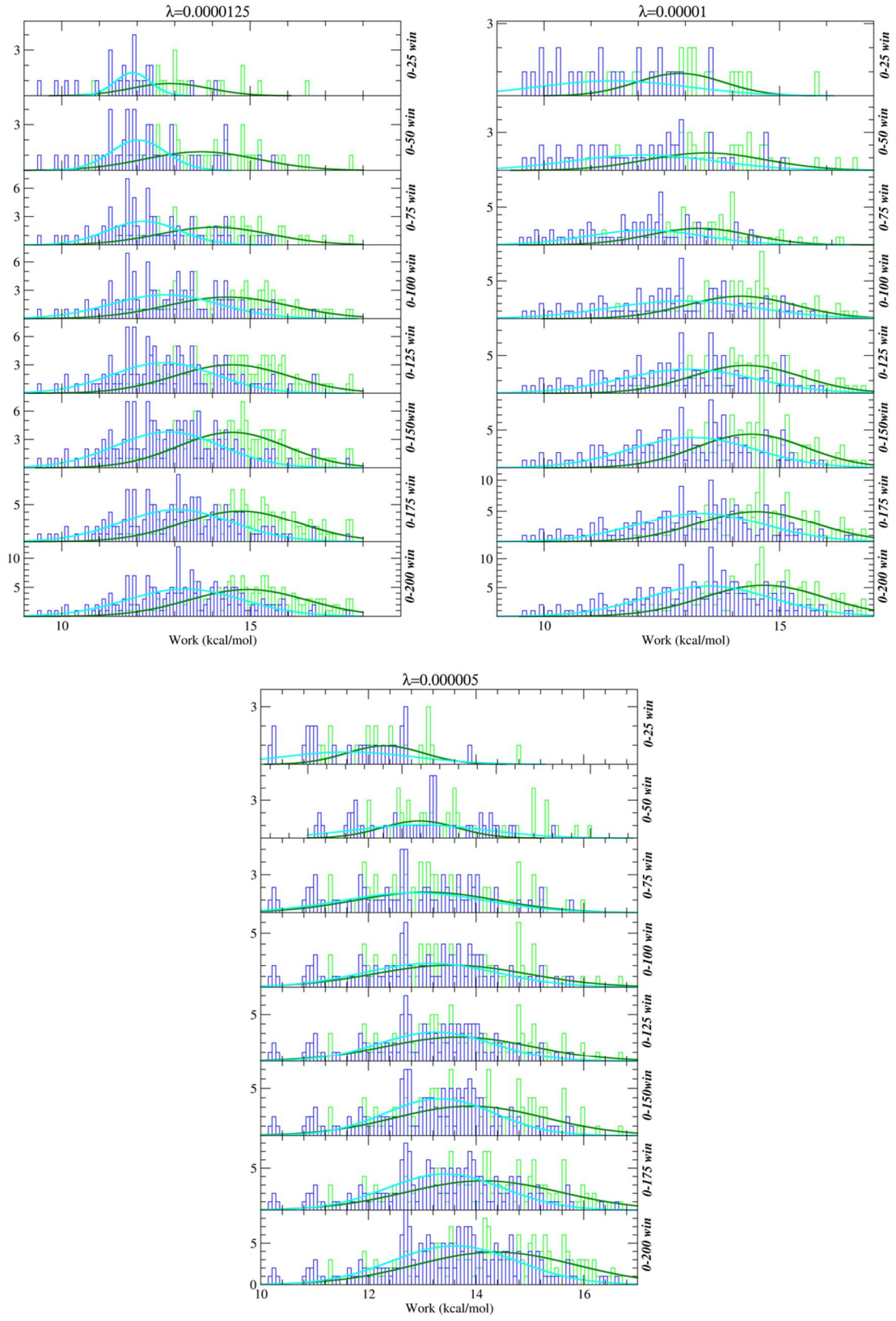


Figure 4. a) $\lambda = 0.0002$, b) $\lambda = 0.0001$, c) $\lambda = 0.00005$ d) $\lambda = 0.000025$ e) $\lambda = 0{,}0000125$, f) $\lambda = 0{,}00001$, g) $\lambda = 0{,}000005$ values, the curves obtained from the curves formed by fitting the histogram data obtained from different number of samples to the gaussian function energy values

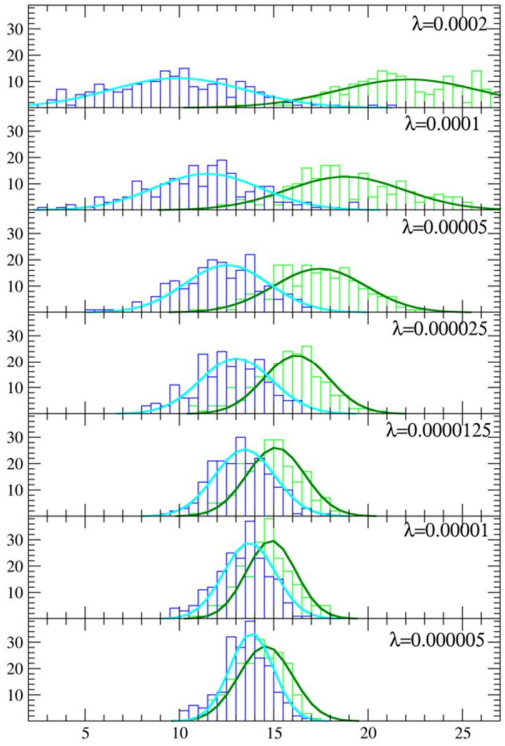


Figure 5. The energy values obtained from the curves formed by fitting the histogram data obtained from 200 samples to the gaussian function for all λ values

Here, 7 different λ values were used to perform the tests of the method. The λ value and the number of steps are given in Table 1. It is clear that the product of these two values will be 1. Forward and backward sample numbers are also given in Table 1. In addition, the computer time run for each sample is shown (time is a simulation on a 12-core computer). The free energy distribution function was created from the free energy obtained over each λ configuration. For the same function, the backward calculation was made, and the energy distribution function of the backward calculation was obtained. These energy distribution functions were plotted for 25, 50, 75, 100, 125, 150, 175 and 200 samples. The aim here is to check whether the method works with the least number of samples. Figure 4 shows these graphs.

Here in Figure 4a, it is seen that the overlap of gaussian curves is the least when λ =0.0002. However, with the increase in the number of samples, it is seen in Figure 4a that the overlaps increase. Figure 5 shows the graph of different λ values for 200 samples. Here, it is seen that the overlap of the gaussian curves with the number of steps changes in the positive direction.

However, what matters to us is not the overlaps, but the intersections of the gaussian curves, as shown in Figure 6. The high energy values for λ = 0.0002 in Figure 6 indicates that we should not take this value. It is seen that other λ values are acceptable especially for 100 samples and beyond (red dashed line). This is an indication that the crooks fluctuation theorem may be valid. The Crooks fluctuation theorem states that the point where the distribution functions intersect corresponds to the free energy. It is approximately the same for the largest and smallest values of the intersection of the distribution functions, and this is in agreement with the free energy difference calculated for the equilibrium state. Also, Figure 6 shows that crooks fluctuation theorem converges extraordinarily fast in the calculation of free energy.

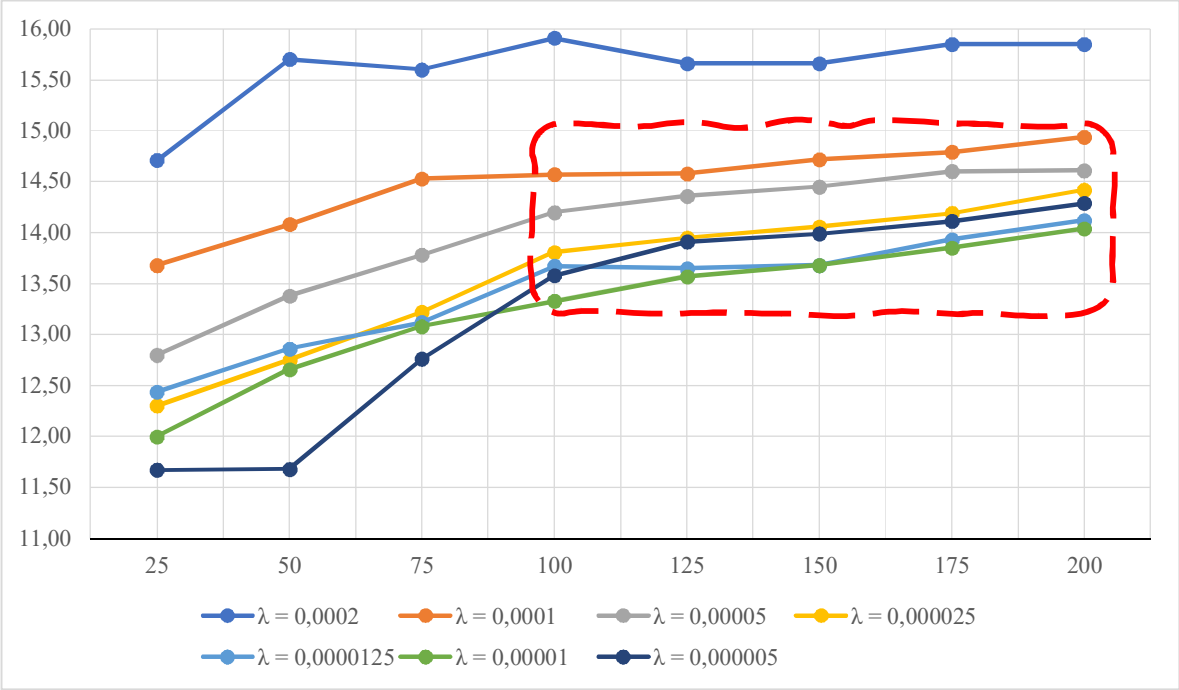


Figure 6. Energy values according to the sample numbers of different λ values

4. CONCLUSION

The passage of ions across the cell membrane is one of the most important fundamental processes of cell biology. Physiological processes such as cell-cell communication and signal transmission, muscle relaxation or contraction begin with the passage of ions or ligands through membrane channels in living organisms. In this process, ion channels try to pass thousands of ions through the cell membrane in a few milliseconds. In such a case, the most important feature that distinguishes biological ion channels from being just a hole in the membrane wall is the selectivity feature. In other words, the ion channel is designed to deliver certain types of ions.

To fully understand the membrane protein system, the free energy behavior of chemical processes must be studied. MD simulations can provide useful information about system mechanism and dynamics, but most of the experimental characteristics can be inferred from the system's free energy information. The free energy of the system can describe how the system is located in a given state. In other words, the free energy difference between the two states is calculated.

In this study, the biological system focused on is the NavAb cell membrane channels that regulate electrical signals called action potentials, information in the nervous system and physiological processes. In the literature, it has been stated that the results about the ion passage through the NavAb channels and the behavior of the selective filter are made by PMF and FEP calculations [33–36]. In this study, it is the development of free energy calculation methods that do not require an equilibrium state free from path-independent convergence problems. The Crooks fluctuation theorem has been tested in biological systems. It is also shown in this way that the Crooks fluctuation theorem converges extraordinarily fast in the calculation of free energy. It should be underlined that the rapid convergence of the free energy calculation is a savings in computation time.

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AUTHOR'S CONTRIBUTIONS

The authors contributed equally.

CONFLICTS OF INTEREST

There is no conflict of interest.

RESEARCH AND PUBLICATION ETHICS

The author declares that this study complies with Research and Publication Ethics.

REFERENCES

[1] J. Payandeh, T. Scheuer, N. Zheng, and W. A. Catterall, "The Crystal Structure of a Voltage-Gated Sodium Channel," Nature, vol. 475, no. 7356, pp. 353–359, July 2011, doi: 10.1038/nature10238.

[2] E. Schrodinger, What is Life? The Physical Aspect of the Living Cell. Cambridge University Press, Cambridge, 1945.

[3] D. Haynes, Biological Thermodynamics. Cambridge University Press, Cambridge, 2008.

[4] E. A. Calzetta, "Kinesin and the Crooks Fluctuation Theorem," Eur. Phys. J. B., vol. 68, no. 4, pp. 601–605, Apr. 2009, doi: 10.1140/epjb/e2009-00113-8.

[5] C. Bustamante, J. Liphardt, and F. Ritort, "The Nonequilibrium Thermodynamics of Small Systems," Physics Today, vol. 58, no. 7, pp. 43–48, July 2005, doi: 10.1063/1.2012462.

[6] F. Ritort, "Nonequilibrium Fluctuations in Small Systems: From Physics to Biology," Adv. Chem. Phys., vol. 137, pp. 31–123, Dec. 2007, doi: 10.1002/9780470238080.ch2.

[7] D. L. Beveridge and F. M. DiCapua, "Free Energy via Molecular Simulation: Applications to Chemical and Biomolecular Systems," Annu. Rev. Biophys. Biophys. Chem., vol. 18, no. 1, pp. 431–492, June 1989, doi: 10.1146/annurev.bb.18.060189.002243.

[8] M. Khabiri, "Computational Investigations of Biomolecular Systems and Comparison with Experiments in Various Environmental Conditions," PhD Dissertation, University of South Bohemia in Ceske Budejovice, Nove Hrady, Czech Republic, 2011.

[9] P. Akhshi, "Molecular Dynamics Simulation Studies of Ion Transport along G-quadruplex DNA Channels," PhD Dissertation, Queen's University, Ontario, Canada, 2013.

[10] E. Buxbaum, Fundamentals of Protein Structure and Function. Springer International, Publishing Switzerland, 2015.

[11] F. Bezanilla, "The Action Potential: From Voltage-Gated Conductances to Molecular Structures," Biol. Res., vol. 39, no. 3, pp. 425–435, Nov. 2006, doi: 10.4067/s0716-97602006000300005.

[12] T. Baştuğ and S. Kuyucak, "Molecular Dynamics Simulations of Membrane Proteins," Biophys. Rev., vol. 4, no. 3, pp. 271–282, Sep. 2012. doi: 10.1007/s12551-012-0084-9.

[13] G. Heinzelmann, T. Baştuğ, and S. Kuyucak, "Free Energy Simulations of Ligand Binding to the Aspartate Transporter GltPh," Biophys. J., vol. 101, no. 10, pp. 2380–2388, Nov. 2011, doi: 10.1016/j.bpj.2011.10.010.

[14] T. P. Straatsma, H. J. C. Berendsen, and J. P. M. Postma, "Free-Energy of Hydrophobic Hydration: A Molecular-Dynamics Study of Noble-Gases in Water," J. Chem. Phys., vol. 85, no. 11, pp. 6720–6727, Dec. 1986, doi: 10.1063/1.451846.

[15] R. Zwanzig, "High-Temperature Equation of State by a Perturbation Method 1. Nonpolargases," J. Chem. Phys., vol. 22, no. 8, pp. 1420–1426, Aug. 1954, doi: 10.1063/1.1740409.

[16] M. Çavuş, "Free Energy Calculation of Ion and Ligand Binding to Membrane Proteins," PhD Dissertation, Gazi University, Ankara, Türkiye, 2013.

[17] G. E. Crooks, "The Entropy Production Fluctuation Theorem and the Nonequilibrium Work Relation for Free Energy Differences," Phys. Rev. E., vol. 60, no. 3, pp. 2721–2726, Sep. 1999, doi: 10.1103/PhysRevE.60.2721.

[18] M. K. P. Ayalasomayajula, "Theoretical Studies of Single Molecule Biophysical Systems and Photochemical Ensembles," PhD Dissertation, California Institute of Technology, Pasadena, California, 2007.

[19] C. Jarzynski, "Nonequilibrium Equality for Free Energy Differences," Phys. Rev. Lett., vol. 78, no. 14, pp. 2690–2693, Apr. 1997, doi: 10.1103/PhysRevLett.78.2690.

[20] B. Hille, Ion Channels of Excitable Membranes, Third Edition. Sinauer Associates, Massachusetts, USA, 2001.

[21] S. Kuyucak and T. Baştuğ, "Physics of Ion Channels," J. Biol. Phys., vol. 29, no. 4, pp. 429–446, Dec. 2003, doi: 10.1023/A:1027309113522.

[22] A. L. Hodgkin and A. F. Huxley, "A Quantitative Description of Membrane Current and Its Application to Conduction and Excitation in Nerve," J. Physiol., vol. 117, no. 4, pp. 500–544, Aug. 1952, doi: 10.1113/jphysiol.1952.sp004764.

[23] D. P. Ryan and L. J. Ptacek, "Episodic Neurological Channelopathies," Neuron, vol: 68, no. 2, pp. 282–292, Oct. 2010, doi: 10.1016/j.neuron.2010.10.008.

[24] W. A. Catterall, "Common Modes of Drug Action on Na1 Channels: Local Anesthetics, Antiarrhythmics and Anticonvulsants," Trends Pharmacol. Sci., vol. 8, no. 2, pp. 57–65, Feb. 1987, doi: 10.1016/0165-6147(87)90011-3.

[25] W. A. Catterall, "Ion Channel Voltage Sensors: Structure, Function and Pathophysiology," Neuron, vol. 67, no. 6, pp. 915–928, Sep. 2010, doi: 10.1016/j.neuron.2010.08.021.

[26] D. Ren, B. Navarro, H. Xu, L. Yue, Q. Shi, and D. E. Clapham, "A Prokaryotic Voltage-Gated Sodium Channel," Science, vol. 294, no. 5550, pp. 2372–2375, Dec. 2001, doi: 10.1126/science.1065635.

[27] R. Y. Yada, R. L. Jackman, and S. Nakai, "Secondary Structure Prediction and Determination of Proteins-A Review," Int. J. Pept. Protein Res., vol. 31, no. 1, pp. 98–108, Jan. 1988, doi: 10.1111/j.1399-3011.1988.tb00011.x.

[28] L. Yue, B. Navarro, D. Ren, A. Ramos, and D. E. Clapham, "The Cation Selectivity Filter of the Bacterial Sodium Channel, NaChBac," J. Gen. Physiol., vol. 120, no. 6, pp. 845–853, Dec. 2002, doi: 10.1085/jgp.20028699.

[29] Y. Zhao, T. Scheuer, and W. A. Catterall, "Reversed Voltage-Dependent Gating of a Bacterial Sodium Channel with Proline Substitutions in the S6 Transmembrane Segment," Proc. Natl. Acad. Sci. USA, vol. 101, no. 51, pp. 17873–17878, Dec. 2004, doi: 10.1073/pnas.0408270101.

[30] W. Humphrey, A. Dalke, and K. Schulten, "VMD-Visual Molecular Dynamics," J. Mol. Graph., vol. 14, no. 1, pp. 33–38, Feb. 1996, doi: 10.1016/0263-7855(96)00018-5.

[31] L. Kale, R. Skeel, M. Bhandakar, R. Brunner, A. Gursoy, N. Krawetz, J. Phillips, A. Shinozaki, K. Varadarajan, and K. Schulten, "NAMD2: Greater Scale Ability for Parallel Molecular Dynamics," J. Comput. Phys. vol. 151, no. 1, pp. 283–312, May 1999, doi: 10.1006/jcph.1999.6201.

[32] A. D. MacKerell, D. Bashford, M. Bellott, R. L. Dunbrack, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, and M. Karplus, "All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins," J. Phys. Chem. B., vol. 102, no. 18, pp. 3586–3616, Apr. 1998, doi: 10.1021/jp973084f.

[33] B. Corry and M. Thomas, "Mechanism of Ion Permeation and Selectivity in a Voltage Gated Sodium Channel," J. Am. Chem. Soc., vol. 134, no. 3, pp. 1840–1846, Jan. 2012, doi: 10.1021/ja210020h.

[34] S. Furini and C. Domene, "On Conduction in a Bacterial Sodium Channel," PLoS Comput. Biol., vol. 8, no. 4, pp. e1002476, Apr. 2012, doi: 10.1371/journal.pcbi.1002476.

[35] H. Qiu, R. Shen, and W. Guo, "Ion Solvation and Structural Stability in a Sodium Channel Investigated by Molecular Dynamics Calculations," Biochim. Biophys. Acta., vol. 1818, no. 11, pp. 2529–2535, Nov. 2012, doi: 10.1016/j.bbamem.2012.06.003.

[36] V. Oakes, S. Furini, and C. Domene, "Voltage-Gated Sodium Channels: Mechanistic Insights from Atomistic Molecular Dynamics Simulations," Curr. Top. Membr., vol. 78, pp. 183–214, Jan. 2016, doi: 10.1016/bs.ctm.2015.12.002.