Final Report

Project title

Study of selectivity ion conduction in Na+ channel by Statistical integral equation theory

By Saree Phongphanphanee

June 2019

Contract No. TRG5780291

Final Report

Project Title

Study of selectivity ion conduction in Na+ channel by Statistical integral equation theory

Saree Phongphanphanee
Department of Materials Science, Kasetsart University

Project Granted by the Thailand Research Fund
Office of the Higher Education Commission and Kasetsart University

Abstract

Project Code : TRG5780291

Project Title : Study of selectivity ion conduction in Na+ channel by Statistical integral equation theory

Investigator : Saree Phongphanphanee

E-mail Address : fscisrph@ku.ac.th

Project Period : 15 July 2015- 14 July 2017

Abstract:

Selectively transport of Na^+ and K^+ across cells provides the electrical signal in cardiac, muscle and nerve cells. To understand the basis of discrimination between Na^+ and K^+ of sodium channel, we investigate the ions distributions in the selectivity filter of voltage gate sodium channel by applying the 3D-RISM theory. The distribution function of water and ions, Na^+, K^+ and Cl^-, in the channel were calculated. Our results show the ion binding at S1, S2 and S4. The PMFs from the implicit ion model show the highest ion selectivity arises at the S2 site. The explicit ion model demonstrated the selectivity arise because the difference of dehydration water molecule between Na^+ and K^+.

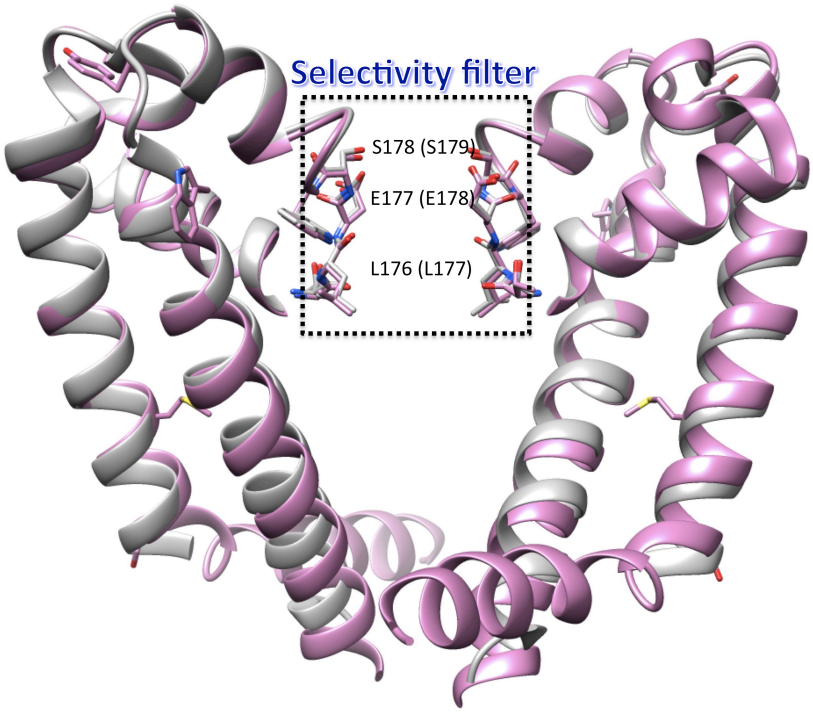
Keywords : 3-5 words

Sodium channel, ion channel, 3D-RISM, selectivity

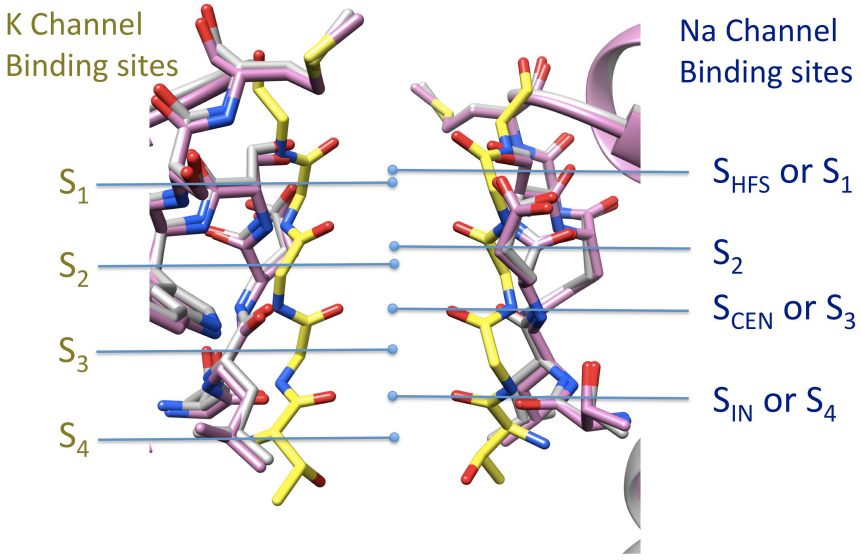
Executive Summary

Introduction

The discrimination of ions transport through cell membrane plays a crucial role in many biological processes in a cell, including neural signal transmission, muscle contraction, homeostasis and cell proliferation.[1-4] The malfunction of ion channels may lead to many diseases, such as epilepsy, ataxia, myotonia and cardiac arrhythmia. The hydrophobic environment in lipid bilayer prevents the transportation of ions across the lipid membrane. To conduct ions into and out of a cell, transmembrane ion channels facilitate the permeation of particular ions through the lipid bilayer membrane along the electrochemical gradient. Many of them are highly selective channels that transport specific ions across the membrane and exclude others species of ion. The understanding of the basis of selectivity of ion channels will pave a way for numerous important applications, including medical treatment, biotechnological development and designing biomimetic materials.



a)



b)

Figure 1 a) Structure of Na channel and b) binding sites of K channel (1J4N in yellow) and Na channel (3RVY in magenta and 5BZB in grey)

Among ion channels, selective Na^+ and K^+ channels have got enormous attention due to their role in physiology. Voltage gate sodium channels (Na_V) are highly selective Na^+ transport over K^+ (about 10-30 fold).[1,2,5] Anaesthetics and various toxins, such as tetrodotoxin and pesticides, are able to bind to the channels and inhibit the ion flow through the cell. Before the discovery of the crystal structure of sodium channel, the molecular studies of ion selectivity in the channel were limited. Chung and co-workers created the pore model of Na channel from the known K channel, which structure was revealed.[6] They estimated the width and length of SF of Na channel and assigned the electric dipole at the channel surface, and applied Brownian dynamic of charge ions in the continuum dielectric liquid. Recently, the first crystal structure Na_V has been revealed in bacteria sodium channel Na_VAb by Catterall and co-authors.[7] The discovery of the structure raised the number of study the molecular mechanics of the ion selectivity of the channel. The channel is composed of four subunits, each contains six transmembrane helices. The helices S5 and S6, P segment form the central pore domain. The motif TLESW from each subunit forms the selectivity filter (SF) of the channel, which is responsible for ion discrimination and bind to ions. Compare to KcsA the selectivity filter of Na_VAb is wider and shorter.[7,8] (Figure 1) Due to the size of the filter, it has hypothesis that sodium ion transport through the channel by fully or partially hydrated ion. From the crystallography study of Payandeh et al., the electron density does not show the binding position of ion at SF.[7] However, by the structure analysis of the pore and comparing with the structure of KcsA, the three binding sites of Na^+ in SF, S_{HFS}, S_{CEN} and S_{IN}, have been propose. (Figure 1b) The site S_{HFS} is bounded by the oxygen of Ser and Glu, which correspond to potassium binding site or S1 of KcsA. However, the positions of the site S_{CEN} and S_{IN} are bounded by carbonyl oxygen of Leu and Thr, the sites correspond to the smaller ions, Li^+ or Na^+, binding site in KcsA, or $S_{2.5}$ and $S_{3.5}$. (Figure 1b) Furthermore, base on the structure of Na_VAb, a number of

molecular simulation studies have also predicted the ion binding sites at the SF.[9-18] However the results from the simulation studies do not coincide. Most of simulation studies indicate that S_{HFS} is located off axis and bound to G177, and the ion is partially hydration at the position. However Carnevale et al. showed the site align on the central axis.[10] The hydration structures of sodium ion at the S_{CEN} are also not concordant: Corry et al., and Furini et al. demonstrate the partially hydrated ion, however Wallace and co-author show the entirely hydration at the site.[12,13] Furthermore, the numbers of binding sites at SF are also not consensus. Some studies show two biding sites SF (S_{HFS} and S_{IN})[10] or three binding sites (S_{HFS}, S_{CEN} and S_{IN}).[12,13] However, a number of simulation studies show the four sites at SF (S1, S2, S3 and S4 in Figure 1b), or there is the binding site between S_{CEN} and S_{IN}.[16,19] The recent crystal structures voltage gate sodium channel, NachBac and NavMs , also supports the four binding sites.[14]

In this work we applied the method of statistical mechanics of liquids theory, or 3D-RISM,[20-22] to study the binding and the solvation of ions in the NavAb and NaBac channel. The theory demonstrates the relations of molecular correlation functions to describe the solvent and solute system. Recently, the theory has been applied to study on various biomolecular systems, especially protein membrane channel such as aquaporin and potassium channel. To verify the ion-binding site at selectivity filter, the three dimensional distribution functions of water and ions obtained by solving the 3D-RISM equation have been investigated. The solvation structures of ions in SF have been analyzed by applying the explicit ion at the binding site.

References

1 Canessa, C. M., Merillat, A. M. & Rossier, B. C. Membrane topology of the epithelial sodium channel in intact cells. *Am J Physiol* **267**, C1682-1690, doi:10.1152/ajpcell.1994.267.6.C1682 (1994).

2 Abriel, H. Roles and regulation of the cardiac sodium channel Na v 1.5: recent insights from experimental studies. *Cardiovasc Res* **76**, 381-389, doi:10.1016/j.cardiores.2007.07.019 (2007).

3 Haugaa, K. H. *et al.* [Cardiac ion channel disorders--diagnosis and treatment]. *Tidsskr Nor Laegeforen* **125**, 2778-2781 (2005).

4 Hille, B. *Ion channels of excitable membranes*. 3rd edn, (Sinauer, 2001).

5 Csanyi, E., Boda, D., Gillespie, D. & Kristof, T. Current and selectivity in a model sodium channel under physiological conditions: Dynamic Monte Carlo simulations. *Bba-Biomembranes* **1818**, 592-600, doi:10.1016/j.bbamem.2011.10.029 (2012).

6 Corry, B., Kuyucak, S. & Chung, S. H. Dielectric self-energy in Poisson-Boltzmann and Poisson-Nernst-Planck models of ion channels. *Biophys J* **84**, 3594-3606, doi:10.1016/S0006-3495(03)75091-7 (2003).

7 Payandeh, J., Scheuer, T., Zheng, N. & Catterall, W. A. The crystal structure of a voltage-gated sodium channel. *Nature* **475**, 353-U104, doi:10.1038/nature10238 (2011).

8 Doyle, D. A. *et al.* The structure of the potassium channel: Molecular basis of K+ conduction and selectivity. *Science* **280**, 69-77 (1998).

9 Boiteux, C., Vorobyov, I. & Allen, T. W. Ion conduction and conformational flexibility of a bacterial voltage-gated sodium channel. *P Natl Acad Sci USA* **111**, 3454-3459, doi:10.1073/pnas.1320907111 (2014).

10 Carnevale, V., Treptow, W. & Klein, M. L. Sodium Ion Binding Sites and Hydration in the Lumen of a Bacterial Ion Channel from Molecular Dynamics Simulations. *J Phys Chem Lett* **2**, 2504-2508, doi:10.1021/jz2011379 (2011).

11 Chakrabarti, N. *et al.* Catalysis of Na+ permeation in the bacterial sodium channel Na(V)Ab. *P Natl Acad Sci USA* **110**, 11331-11336, doi:10.1073/pnas.1309452110 (2013).

12 Corry, B. & Thomas, M. Mechanism of Ion Permeation and Selectivity in a Voltage Gated Sodium Channel. *J Am Chem Soc* **134**, 1840-1846, doi:10.1021/ja210020h (2012).

13 Furini, S. & Domene, C. On Conduction in a Bacterial Sodium Channel. *Plos Comput Biol* **8**, doi:ARTN e100247610.1371/journal.pcbi.1002476 (2012).

14 Naylor, C. E. *et al.* Molecular basis of ion permeability in a voltage-gated sodium channel. *Embo J* **35**, 820-830 (2016).

15 Stock, L., Delemotte, L., Carnevale, V., Treptow, W. & Klein, M. L. Conduction in a Biological Sodium Selective Channel. *J Phys Chem B* **117**, 3782-3789, doi:10.1021/jp401403b (2013).

16 Ulmschneider, M. B. *et al.* Molecular dynamics of ion transport through the open conformation of a bacterial voltage-gated sodium channel. *P Natl Acad Sci USA* **110**, 6364-6369, doi:10.1073/pnas.1214667110 (2013).

17 Zhang, X. *et al.* Crystal structure of an orthologue of the NaChBac voltage-gated sodium channel. *Nature* **486**, 130-U160, doi:10.1038/nature11054 (2012).

18 Zhang, X. *et al.* Analysis of the selectivity filter of the voltage-gated sodium channel NavRh. *Cell Res* **23**, 409-422, doi:10.1038/cr.2012.173 (2013).

19 Ulmschneider, M. B., Bagneris, C., McCusker, E. C., Ulmschneider, J. P. & Wallace, B. A. Microsecond Molecular Dynamics Simulations of the Open State Structure of a Bacterial Voltage-Gated Sodium Channel Reveal Mechanisms of Ion Selectivity and Conduction. *Biophys J* **104**, 132a-133a (2013).

20 Hirata, F. *Molecular theory of solvation*. (Kluwer Academic, 2003).

21 Yoshida, N., Imai, T., Phongphanphanee, S., Kovalenko, A. & Hirata, F. Molecular recognition in biomolecules studied by statistical-mechanical integral-equation theory of liquids. *J Phys Chem B* **113**, 873-886, doi:10.1021/jp807068k10.1021/jp807068k [pii] (2009).

22 Ruankaew, N., Yoshida, N., Watanabe, Y., Nakano, H. & Phongphanphanee, S. Size-dependent adsorption sites in a Prussian blue nanoparticle: A 3D-RISM study. *Chem Phys Letts* **684**, 117-125, doi:10.1016/j.cplett.2017.06.053 (2017).

Objective

1. To investigate the binding of ions in the selectivity filter of Na^+ channel.
2. To investigate the solvation structure of ions in the selectivity of Na^+ channel.
3. To understand the relation of solvation and selectivity of ion in Na^+ channel.

Research methodology

To perform 3D-RISM calculations of the Na^+ channel, we used the crystal structures of NavAb (PDB ID: 3RVY) and (PDB ID: 5BZB). The protein channels were considered as the solute immersed in an electrolyte solution, or solvent, at infinite dilution. The dielectrically consistence reference interaction site model (DRISM) was performed to obtain the solvent-solvent correlation function. The solvents used in this study were NaCl and KCl at 0.1 M. The potential parameters set for water and ions are TIP3P and modified OPLS for Amber. The dielectric constant and temperature in the calculations were 78.5 and 298 K. Number of grid and spacing in the DRISM calculations were 8092 and 0.02 A. To solve DRISM and 3D-RISM, the equations were

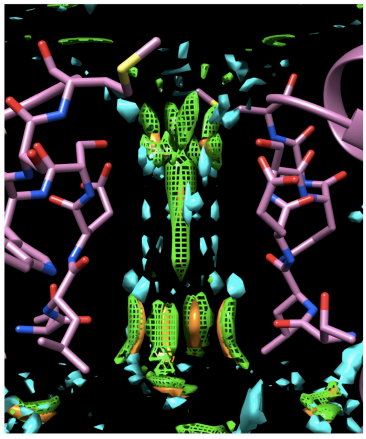
solve in couple with the KH-closure. The potential parameters of the solute, protein channel, were AMBER99. The three-dimensional distribution functions (3D-DFs) of solvents were obtained from the solution of 3D-RISM equation. To calculate PMF, we averaged the 3D-DF along channel axis and take logarithm on the averaged distribution function. [] The solvated structure of ion at the SF was investigated by applying an explicit ion, Na^{+} or K^{+}, at the ion-binding site and calculating the 3D-DF of water in the channel. The 3D-RISM equations were numerically solved on a grid of 256^{3} points in a cubic supercell of 128 A^{3}.

Results and Discussions

The figure 2 represents the 3D-DF of water, Na^+, K^+ and Cl^- inside the channels of 3RVY and 5BZB. Consider the anion, there is a gap of distribution of Cl^- in both channels at Glu (E177 of 3RVY and E178 of 5BZB) and Ser (S178 of 3RVY and S179 of 5BZB), Figure 2b and 2c. The anion is excluded from the region of the channels due to the strong negative environment created by the four Glu. For the cations, the 3D-DFs of Na^+ and K^+ were similar to each other; their peaks located at almost same position. Moreover, Na^+ has higher distribution at the selectivity filter (SF) than K^+. The figure 2d illustrates the position of the peaks of distribution of the cations at the selectivity filter and their corresponding binding sites in 3RVY and 5BZB. The peaks of distribution of cations at SF in 3RVY are consistent to the three binding sites, S1(S_{HFS}), S2 and S4(S_{IN}), whereas the peaks in 5BZB are agreeable to the four binding sites, S1(S_{HFS}), S2, S3(S_{CEN}) and S4(S_{IN}). The four peaks of cations corresponded to binding site S1 for both channels (3RVY and 5BZB) are at the off central axis and each is bounded to a carboxylate oxygen atom of Glu (E177 of 3RVY and E178 of 5BZB) and a hydroxyl oxygen atom of Ser (S178 of 3RVY and S179 of 5BZB). Whereas it has the highest peak of cations located the central axis at S2 for the both 3RVY and 5BZB. We found the four peaks of distribution of cations appear at S3(S_{CEN}) only for 5BZB, but not for 3RVY. Each of the peaks of ion is bounded to a carbonyl oxygen atom of Leu (L177 of 5BZB) and located at off channel axis. Similar to the S3 site of 5BZB, four peaks of distribution of cations at S4(S_{IN}) are bounded to a carbonyl oxygen atom of Thr (T175 of 3RVY and T176 of 5BZB) in both channels. The binding site S3(S_{CEN}) and S4(S_{IN}) are difference to the plane biding sites in KcsA, which are the binding sites for Li^+ or small alkali ion. The plain sites in KcsA are at the center of the plane form by four carbonyl oxygens, however, the S3(S_{CEN}) and S4 site are located at the off axis.

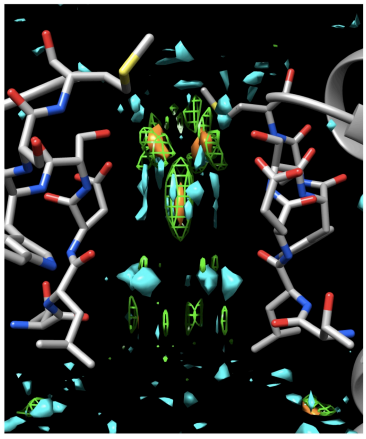
For water distribution in SF, we found that several peaks of water surround the peak of cations at S2 (Figure 2a) for both channels. It indicates that a cation at the binding site has fully hydration structure. At others binding sites, the peaks at the sites are bound to an oxygen atom of the channel.

3RVY



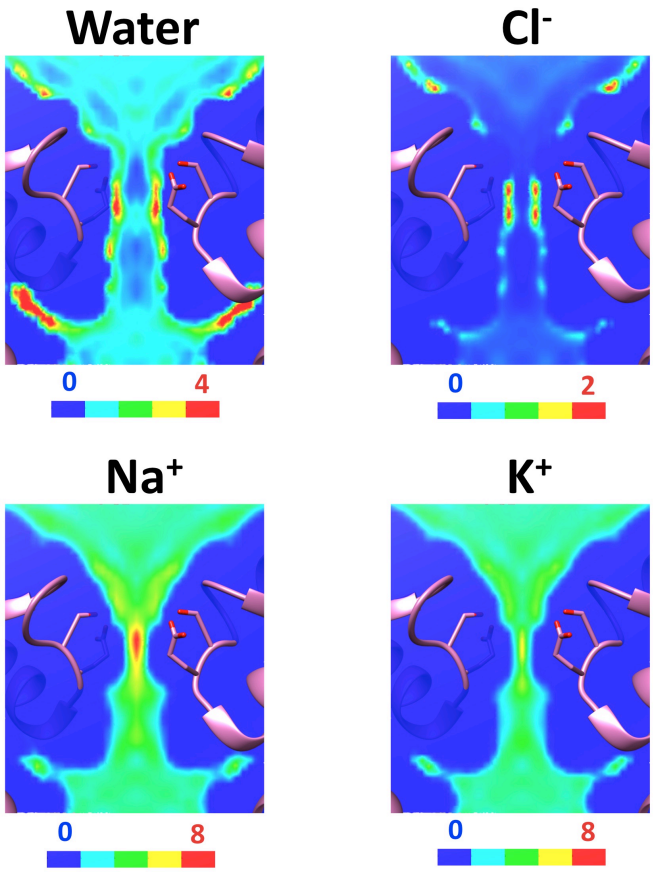
Blue: Water (g>5)
Green: Na+ (g>5)
Orange: K+ (g>5)

5BZB

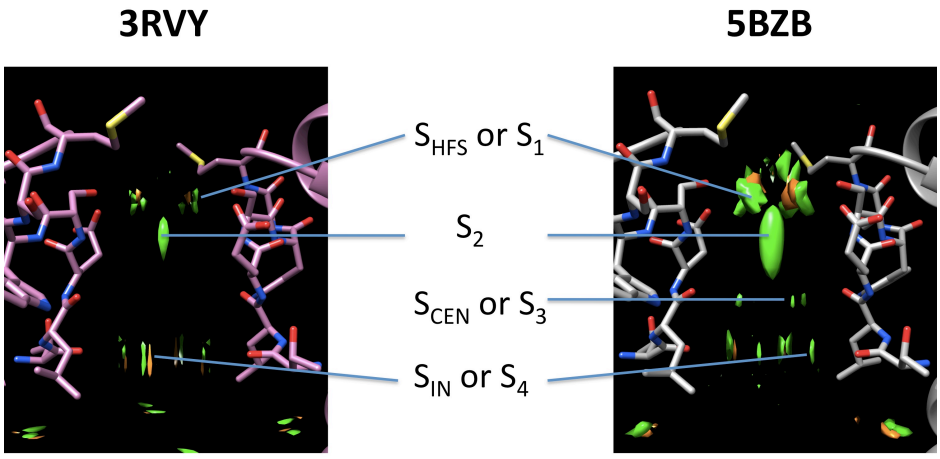


Blue: Water (g>5)
Green: Na+ (g>4)
Orange: K+ (g>4)

a)



b)



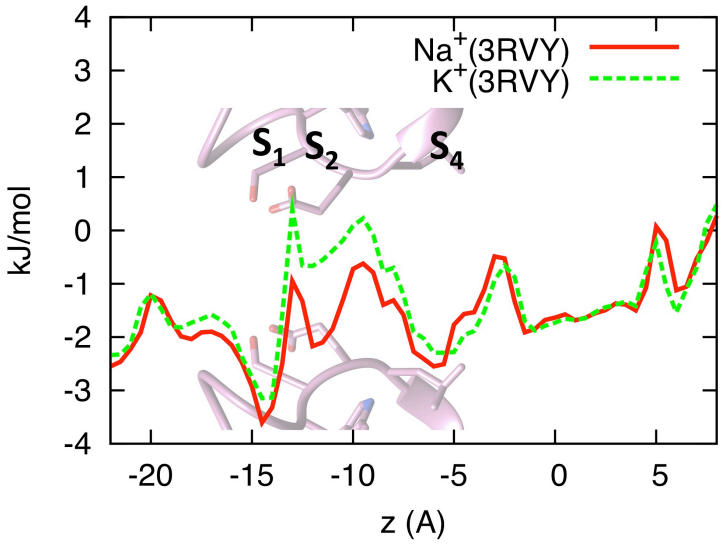
Green: Na+ (g>7)
Light brown: K+ (g>6)

Green: Na+ (g>4)
Light brown: K+ (g>3.5)

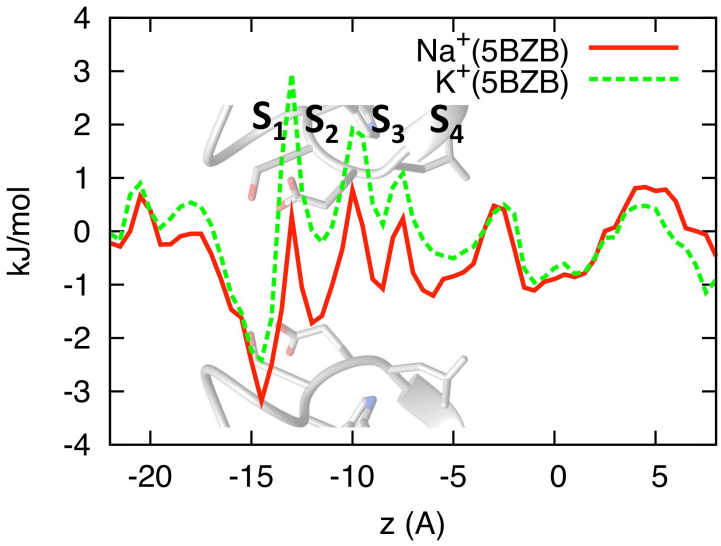
c)

Figure 2 a) 3D-DFs of water and cations, b) contour plot of 3D-DFs of water and ions (Cl^-, Na^+ and K^+) in 3RVY, c) contour plot of 3D-DFs of water and ions (Cl^-, Na^+ and K^+) in 5BZB, and d) peaks of 3D-DFs of Na^+ and K^+ correspond to the binding sites.

We also have calculated PMFs of ions along the channel axis of 3RVY and 5BZB by averaging the 3D-DF along the channel axis. At SF of both channels, the Figure 3a and 3b show the higher of PMF of Na^+ over K^+, it indicates the selectivity arise in this area. The minima in PMFs correspond to the ion binding site at S1, S2, S3 and S4. Our results show the highest difference in PMF between two ions is at site S2 and S3. This indicates the selective area of the channel is located at the region.



a)



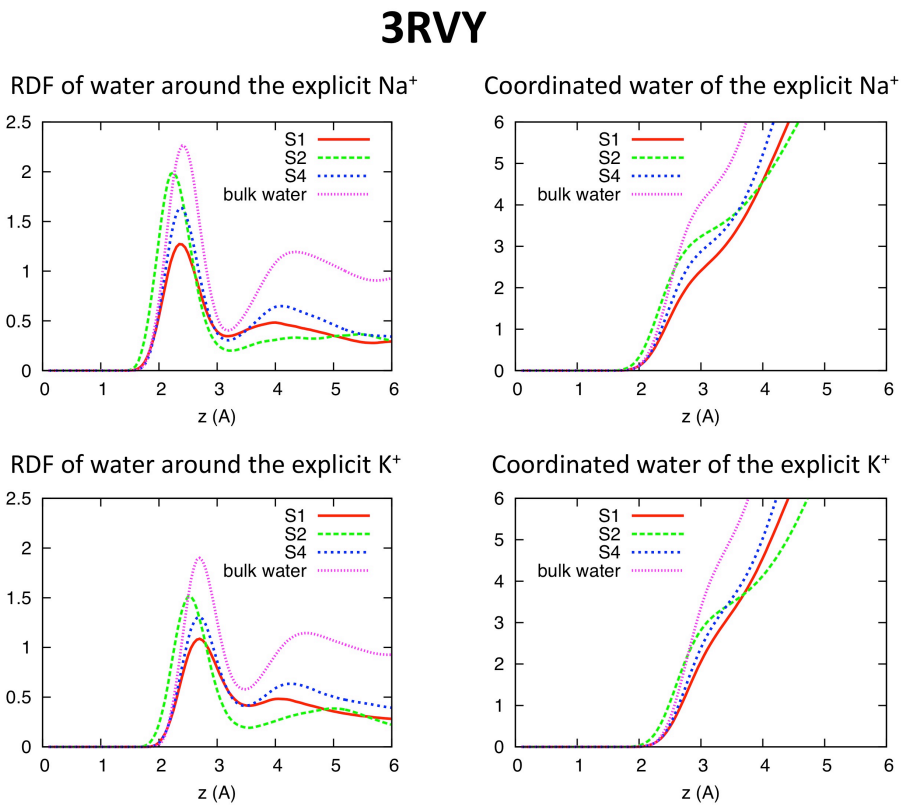
b)

Figure 3 PMF of ions in SF of a) 3RVY and b) 5BZB

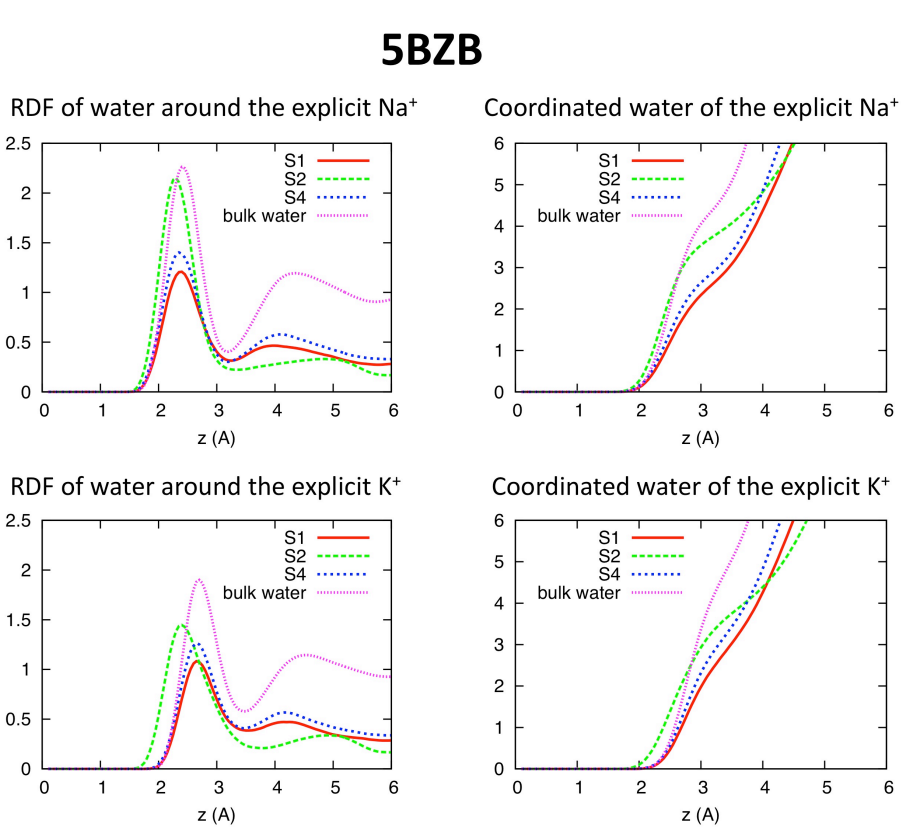
Explicit ion model

To analyse the basis of selectivity of the channel, we applied an explicit ion at its binding site and investigate its solvated structure at selectivity filter. The ion was placed at the highest peaks around the SF, or its binding site, shown in figure 2d. The 3D-RISM equation was solved to get the distribution function of water in channel with the explicit ion. The radial distribution function (RDF) of water around an explicit ion was calculated by angular averaging of 3D-DF around the ion, and the number of coordinated water was calculated by integrating the RDF. The water coordination number of ions at bulk and binding sites were reported in the table 1. To transport an ion through the channels, the ion move from the bulk to the site S1(S_{HFS}), approximately two water molecules are dehydrated for both ions. The loss of coordinated water is

compensated by two oxygens from Glu and Ser. Then the total coordination number of ions is close to the bulk. At S2, the site is at the central axis and the ion is bounded to only water to form fully hydration structure. Furthermore, Na^+ and K^+ have the similar water coordination number of at S2 for both channels. The coordinated water number of the ion at the site are less than that of the bulk, the numbers of dehydrated water molecule of Na^+ and K^+ are 0.9 and 1.6 for 3RVY, and 0.5 and 1.0 for 5BZB. As can be seen from the results, Na^+ has lower dehydration number at S2 site that K^+ for both channels. When the ion move to S3 (5BZB) and S4 (3RVY and 5BZB), it bound to a carbonyl oxygen atom at off axis of the channel, and form partially hydration structure. The water coordination number of these sites are less than that of the site S2. Our results indicated that the highest difference of dehydration number of S2 is the cause of ion selectivity in Na channel, this are in accord to the PMF from implicit ion model in Figure 3.



a)



a)

Figure 4 RDF of water and coordinated water around the explicit ion at binding sites of a) 3RVY and b) 5BZB.

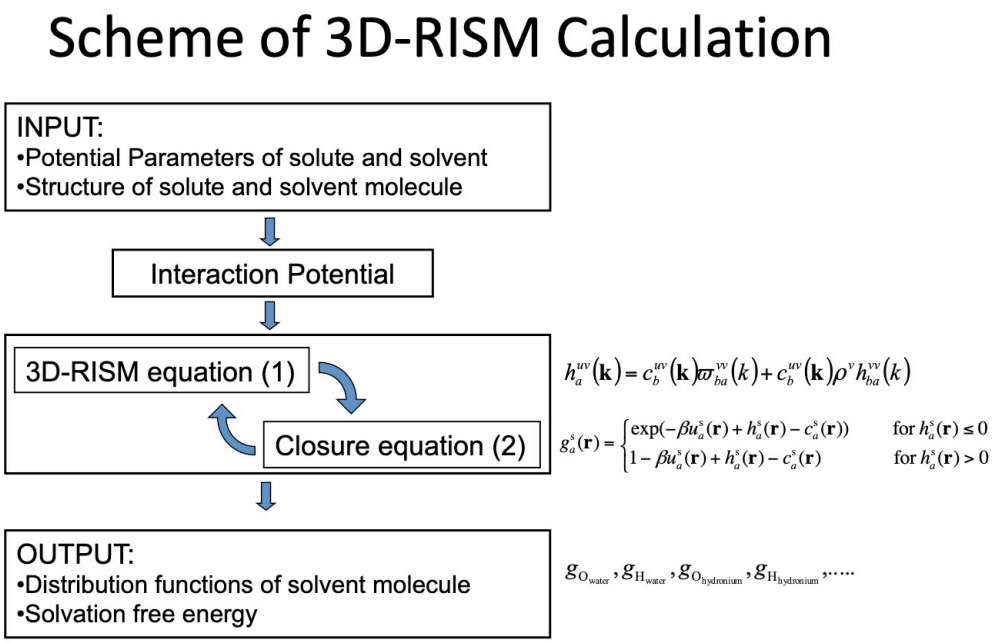


Figure 5 Scheme of calculation

Table 1 Number of coordinated water of explicit ion at BS and bulk

		S1 (S_{HFS})	S2	S3 (S_{IN})	S4 (S_{CEN})	Bulk
Na^+	3RVY	2.7	3.5	-	3.1	4.4
	5BZB	2.6	3.9	3.2	2.9	
K^+	3RVY	3.4	3.5	-	3.5	5.1
	5BZB	3.1	4.1	3.4	3.3	

Conclusions

The distribution function of water and ion in the sodium channel were calculated by 3D-RISM theory. The binding sites appears at S1, S2 and S4. The PMF from the implicit ion model shows the ion discrimination arise at the selectivity filter, and highest at S2. The explicit ion model demonstrate water coordination of around explicit Na+ is changed along the SF, whereas that of K+ is not change. The loosing in number of hydration and PMF of Na^+ and K^+ at S2 site are consistent. Then our finding indicates that greater dehydrated water molecules of K than that of Na might be the basis of the selectivity.

Output

International Journal Publication

Preparing for submission in the title of "*Molecular basis of ion selectivity in sodium channel by the statistical mechanics of liquid.*"

International conference

1. The 10th International Symposium of the Protein Society of Thailand, 15-17 July, 2015, Bangkok, Thailand, on the title *"Ion distribution in ion channels study by the statistical mechanics of liquid".*
2. The 4th Symposium Studying the Functional of Soft Molecular Systems by the Concerted Use of Theory and Experiment, 27-28 October 2016, Japan, on the title of "*A 3D-RISM Study of Ion Selectivity of Sodium Channel*."