Recent Advances in Computational Studies on Influenza A Virus M2 Proton Channel

Jing-Fang Wang*[,1,2,3] and Kuo-Chen Chou*[,3]

[1]*Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China;* [3]*Shanghai Center for Bioinformation Technology, Shanghai 200235 China;* [3]*Gordon Life Science Institute, 13784 Torrey Del Mar Drive, San Diego, California 92130, USA*

Abstract: The matrix protein 2 of the influenza A virus (M2 or AM2) is one of the important components of the viral membrane. This protein can form a proton channel in the viral envelope. Owing to its ability to regulate the surrounding pH in endosome, this protein is an attractive target for drug design against influenza A virus. In this minireview, we summarized the current progresses in computational approaches for studying the M2 proton channel. The attention is focused on how protons are conducted through the M2 channel, and how adamantane-based drugs inhibit the channel, as well as how the drug resistance occurs, in hope to further stimulate the in-depth studies of this important area, both experimentally and theoretically.

Keywords: Influenza A virus, M2 proton channel, molecular modeling, molecular dynamics, proton conductance, drug binding site, drug inhibition, drug resistance.

INTRODUCTION

The matrix protein 2 of the influenza A virus (also called M2 or AM2), together with the matrix protein 1 (M1), is encoded by the 7th RNA segment of influenza A virus [1-3]. Although the M2 protein is in much lower abundance compared with hemagglutinin (HA) or neuraminidase (NA), this protein still acts as one of the most important components in the life cycle of influenza A [4]. The monomer of the M2 proton channel has 97 residues [5,6], comprising an extracellular N-terminal domain (residues 1-23) directed toward the outside of influenza A viruses, a conserved transmembrane domain or region (residues 24-46), as well as an intracellular C-terminal domain (residues 47-97). It is proved that this protein can form a channel in lipid bilayers by four parallel monomers with inter-monomer disulfide bonds at Cys17 and Cys19 [7,8]. Further biology studies show that the M2 proton of influenza A virus can function as a proton channel to equilibrate pH values across the viral membrane during cell entry and across the trans-Golgi membrane of infected cells during viral maturation [9].

In endosome, the viruses of influenza A will go through two important pH alterations: from the extracellular pH to the early endosomal pH (~6), and from the early endosomal pH to the late endosomal pH (~5) [10]. Both pH alterations play crucial roles in the viral life cycle [11], especially the latter alteration that is critical for the membrane fusion for its ability to activate HA to catalyze the fusion of the viral envelope with the endosomal membrane. Before this process, the M2 channel can be activated by the low pH surrounding to conduct protons across the viral envelope, leading to the acidification of the viral interior [11,12]. Thus, as an essential component of the viral envelope, the M2 protein has been a key target for drug design against influenza A virus.

Recently, high resolution structure of the M2 proton channel from influenza A virus has been simultaneously released by solution NMR spectroscopy (Fig. **1A**) [5] and X-ray crystallography (Fig. **1B**) [13]. Both structures accord with the basic, but differ in detailed information. The solution NMR structure of the M2 channel was determined at pH 7.5, and adopted a closed state conformation bound to the drug rimantadine [5]. Also, the solution NMR structure covers substantially more region of the entire M2 protein, including an unstructured N-terminus (residues 18-23), a transmembrane domain (residues 24-46), a flexible loop (residues 47-50), and a C-terminus amphipathic helix (residues 51-60). In the transmembrane domain, the helices form a four-helix bundle with a left-handed twist angle [14,15] of about 23 degrees. The transmembrane helices are tightly packed at the N-terminus, slightly splaying out toward the C-terminus. As a result, the channel is constricted to 1.4-1.7 Å for tightly packing of the imidazole of His37 and the indole of Trp41, narrowing the entrance and restricting waters from penetrating the channel. Thus, the solution NMR structure is believed to be a completely closed conformation. Additionally, hydrogen bonds can be detected between the indole amine of Trp41 and the carboxyl carbon of Asp44 in the adjacent subunit [11,16,17], which can further lock the channel gate in this closed conformation [18].

Rather than 43 residues per chain as in the NMR structure [5], the crystal structure contains only 25 residues per chain [13]. In the crystal structure, the transmembrane

*Address correspondence to these authors at the Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China;
Tel: +86-131-6728-2637; Fax: +86-21-3420-4573;
E-mails: jfwang8113@sjtu.edu.cn, kcchou@gordonlifescience.org

1875-5607/12 $58.00+.00

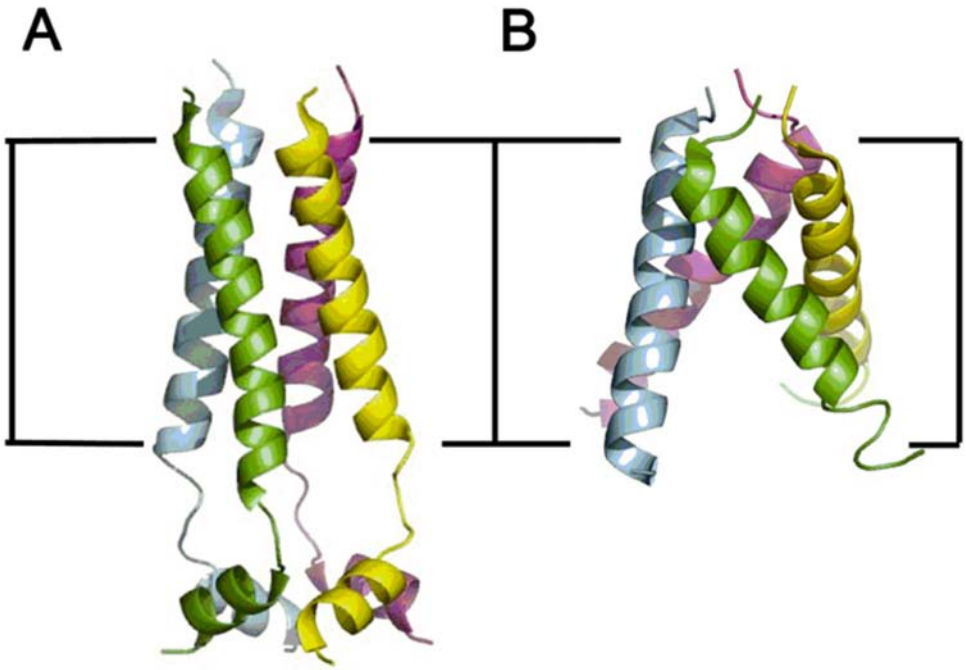

Fig. (1). High resolution structures of the M2 proton channel. (**A**) Solution NMR structure covering 43 residues (res.18-60) determined at pH 7.5 (2rlf.pdb). (**B**) Crystal structure covering 25 residues (res.22-46) determined in beta-octylglucoside detergent at pH 7.3 (3c9j.pdb).

helices are tightly packed at the N terminus, splaying outward to the C-terminus at an average angle of nearly 35 degrees (Fig. **1B**). As a consequence, the distance between the indoles of Trp41 from adjacent transmembrane helices is about 9.5 Å apart. It is identified by mutagenesis studies that two pore-lining residues, the imidazole of His37 and the indole of Trp41, are key factors for the channel function of the M2 protein. In this case, the C-terminus region is in an open state without obvious structure features to support proton gating and selection. One possible explanation is that the crystal structure does not include a structural C-terminal domain, which have demonstrated to be a crucial component for a stable tetramer formation, native-like conductance, and sensitivity to adamantane-based drugs according to the functional studies [19,20]. Thus, due to losing the C-terminus region, the crystal structure is clearly inconsistent with the closed channel conformation at 7.3 pH [18].

Subsequently, the high-resolution NMR structure for the influenza B proton channel (Fig. **2**), abbreviated as BM2, has also been successfully determined recently [21]. The knowledge of 3-D (dimensional) of the M2 channel is very important for really understanding the proton-conducting mechanism among others such as drug binding/inhibition, and drug resistance. Before the high resolution 3-D structure for the M2 channel [5] is available, a number of structural models of the transmembrane region were constructed based on the information from biochemical experiments [22-24], site-directed infrared dichroism [25], and solid-state NMR spectroscopy [26-30]. Meanwhile, computational and theoretical studies were also performed on the M2 proton channel in hopes to acquire useful insights into the proton-conducting mechanism at the atomic level.

Many evidences have indicated that the computational and theoretical studies, such as molecular modeling [31-48], molecular docking [3,49-56], molecular dynamics simulations [43,50,57-65], quantum mechanics calculation [39,66], pharmacophore modeling [67-69], QSAR [16,70-75], and various structural bioinformatics approaches [76], can provide useful information in a timely manner to stimulate both basic research and drug development [37,57,77]. Particularly, the recent successful determination of the high-resolution 3-D structure for the almost complete M2 channel [5] has not only greatly stimulated many in-depth computational investigations into the proton channel and its action mechanism but also provided a solid foundation for conducting these studies. In view of this, the present minireview was initiated in an attempt to summarize the progresses in this regard, with the focuses on the detailed proton-conducting mechanism of the M2 membrane channel, as well as its action mechanisms on drug binding/inhibition and drug resistance.

PROTON CONDUCTANCE

Proton conductance in the M2 channel plays a crucial role in the viral life cycle. When the pH is low in endosome, the M2 channel can be activated leading to the acidification of the viral interior by conducting protons across the viral envelope [12,78]. The acidification of the viral interior can further weaken the electrostatic interaction between M2 and ribonucleoprotein complexes so that the subsequent membrane fusion can release the uncoated ribonucleoproteins into the cytosol [79]. Due to the dynamic exchange with water, buffers, and titratable groups of lipids and proteins, proton conductance cell membranes employ different mechanisms from those adopted by other ion channels. In most biological

systems, the protons are proposed to move in water by hopping from one water molecule to another along water chains of hydrogen-bonded water molecules. Such mechanism has been proposed those channels, such as gramicidin A proton channel and bacteriorhodopsin [80-84].

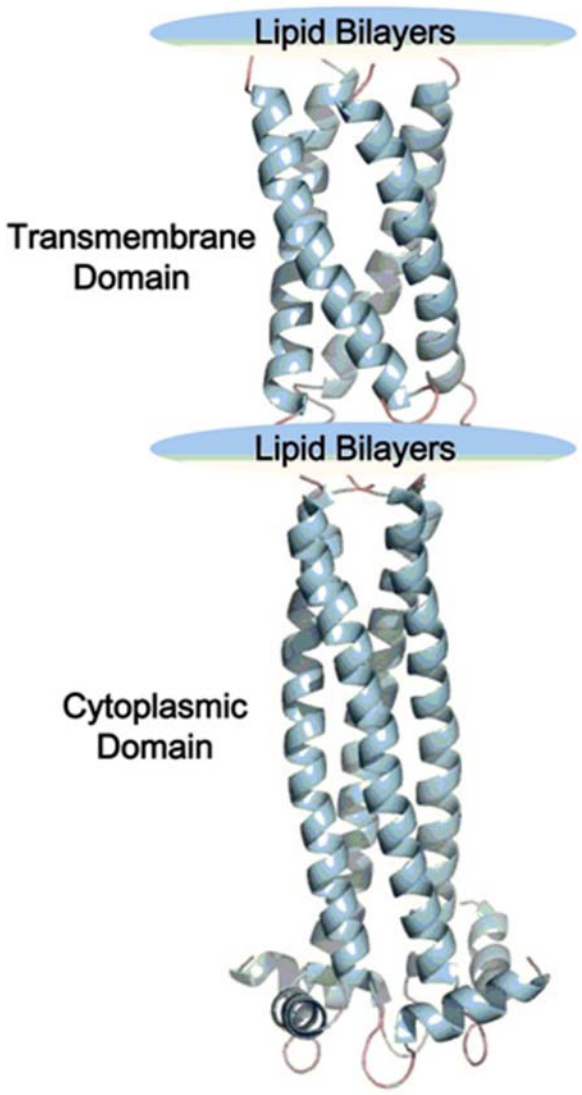

Fig. (2). A ribbon drawing of the BM2 structure. It was derived from a combination of the pdb codes of 2KIX and 2KJ1 (the former is for the channel domain, while the latter for the cytoplasmic domain). Reproduced from [21] with permission (3c9j.pdb).

To check if the M2 channel uses a hydrogen-bonded water chain to conduct protons, a series of computational and theoretical works has been done. As aforementioned, two pore-lining residues, His37 and Trp41, are the key factors for the channel function of the M2 protein. Thus, it is believed that His37 and Trp41 are gating residues for the M2 protein, based on which it was suggested that protonated His37 might result in the opening of the channel gate, allowing protons across the C-terminus domain [85]. Strong evidences gained from mutagenesis studies showed that mutations at position 37 could greatly alter the conductance behavior of the M2 proton channel [86-89]. Further computational studies have also confirmed that for the closed state of the M2 channel, the imidazole side chains of His37 are directed toward the lumen, thereby occluding the pore and forming a channel gate [90-96]. Based on these findings, two possible conductance mechanisms, the gating mechanism and the shuttle mechanism, are proposed.

In the gating mechanism, the imidazole moiety of His37 can accept an additional proton with positive charge in a low pH surrounding. However, the accepted proton will not be immediately released back to the pore water. Instead, it binds to the histidine residue in a manner of dynamic equilibrium. Thus, due to the electrostatic repulsion between the positive charges in the M2 tetramer, the side chains of the histidine imidazole moiety keep away from each other, so as to open the occluded pore and allow the pore water to form a continuous proton-conductive water chain. Recently, such a gating mechanism was supported by many computational and theoretical studies [97-108]. In the shuttle mechanism, His37 is directly involved in a proton relay. Accordingly, when the M2 channel is activated, in contrast to the gating mechanism, a hydrogen atom will be released back to the pore water while the side chain of the His 37 imidazole acquires a proton to form a bi-protonated intermediate.

DRUG BINDING AND INHIBITION

In the crystal structure of the transmembrane region (3c9j.pdb), adamantane-based drugs are proposed to binding in the channel pore binding site, around Ser31 and Gly34 (Fig. **3A**). The hydrophobic adamantly group is coordinated to the hydroxyls of Ser31, suggesting a direct pore-blocking mechanism. In the solution NMR structure (2rlf.pdb), adamantane-based drugs are proposed to locate on the lipid-facing binding site formed by Trp41, Ile42, as well as Arg45 from one transmembrane helix, and Leu40, Leu43, as well as Asp44 from the adjacent transmembrane helix (Fig. **3B**). Such a drug location gives an indication that the drug inhibits the M2 proton channel allosterically by stabilizing the closed conformation.

To study the drug binding and inhibition mechanism, many good attempts have been made. In 2008, Intharathep *et al.* used computational approach for the first time to study the channel pore and lipid-facing binding models of the M2 proton channel [109]. Although neither the crystal structure of the transmembrane region (3c9j.pdb) nor the solution NMR structure (2rlf.pdb) was used in their study, they constructed the complexes of the M2 channel with the adamantane-based drugs in both channel pore and lipid-facing binding models. Via simulating 7 possible protonation states of His37, they found that the water density in the M2 channel was notable reduced by the adamantane-based drugs in both channel pore and lipid facing models. Based on this finding, they suggested that both binding models might simultaneously exist in the M2 channel.

Meanwhile, using the molecular docking approach, Huang *et al.* [11] performed an in-depth analysis, further validating the NMR structure and its action mechanism.

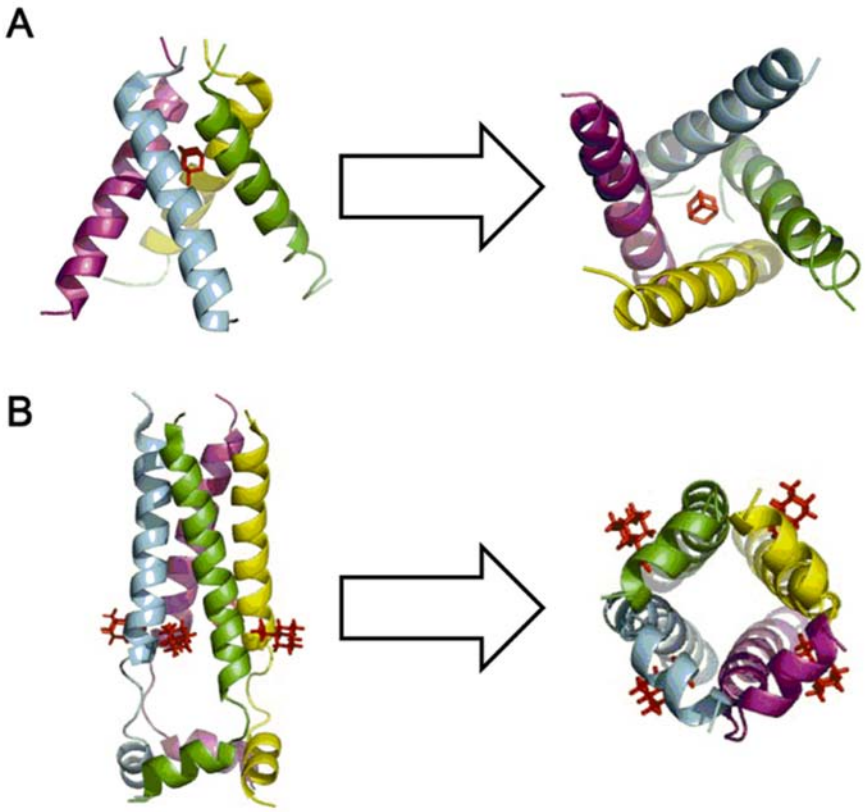

Fig. (3). Proposed adamantane-based drug binding site on the M2 channel. (**A**) The channel pore binding site derived from the crystal structure [13]. (**B**) The lipid-facing binding site derived from the high-resolution NMR structure [5].

Stimulated by the findings in [11] and based on the NMR structure [5], Wei *et al*. [16] and Du *et al*. [17] respectively proposed a new strategy to deal with the drug-resistance problems and developing new effective drugs against H5N1 avian influenza virus.

Subsequently, Du *et al*. [18] performed a detailed structural analysis on the crystal structure and solution NMR structure of the M2 channel. Using the flexible docking and energy-based scoring functions, they calculated the binding affinities of the M2 channel with adamantane-based drugs in both the channel pore and lipid-facing binding models were calculated and found that the lipid-facing binding model had much higher binding affinities than the channel pore binding model.

In a separate work, Wang *et al*. [51] also performed a structural comparison between the channel pore and lipid-facing binding models based on the homology model of the M2 channel from the H1N1 swine virus. In their study, the flexible docking and classical molecular dynamics simulations were employed on the complexes of the M2 channel with the adamantine-based drugs. They found that, quite consistent with the report in [18], the binding free energies are more favorable to the lipid-facing binding model as proposed in [5].

Based on these drug binding models, some experimental and computational works have been done to study the inhibitors against the M2 channel. In 2009, Wei *et al*. [16] constructed a fragment based QSAR model for the adamantane-based M2 inhibitors. They used 34 M2 channel inhibitors against H3N2 influenza A virus to construct the QSAR model. Based on fragment-based analyses, they found that fragment F_2 in adamantane-based drugs should be focused to conduct the adamantane-based antiflu drug design. In 2010, Zarubaev *et al*. [110] synthesized a series of azolo-adamantanes against influenza A virus. They used both chemical and biological experiments to confirm the aforementioned computational results, and developed a new QSAR model based on both experimental and computational results.

DRUG RESISTANCE

Adamantane-based drugs amantadine and rimantadine are firstly developed to fight against influenza A virus, targeting the M2 proton channel. However, after its approval for clinical treatment of influenza A virus, the drug resistance has gradually increased. By now, nearly 100% H3N2 subtype of avian influenza virus has resistance to the adamantane-based drugs in Asian countries, and more than 15.5% of H1N1 viruses are resistant to the adamantane-based drugs. According to the report of Suzuki *et al*. [111], the drug resistance to the adamantane-based drugs is associated with the single point mutations L26F, V27A, A30T, S31N, G34E, and L38F [112]. It has been shown according to the statistical data of the clinical samples that for the resistant viruses, nearly 80% of substitutions occur at

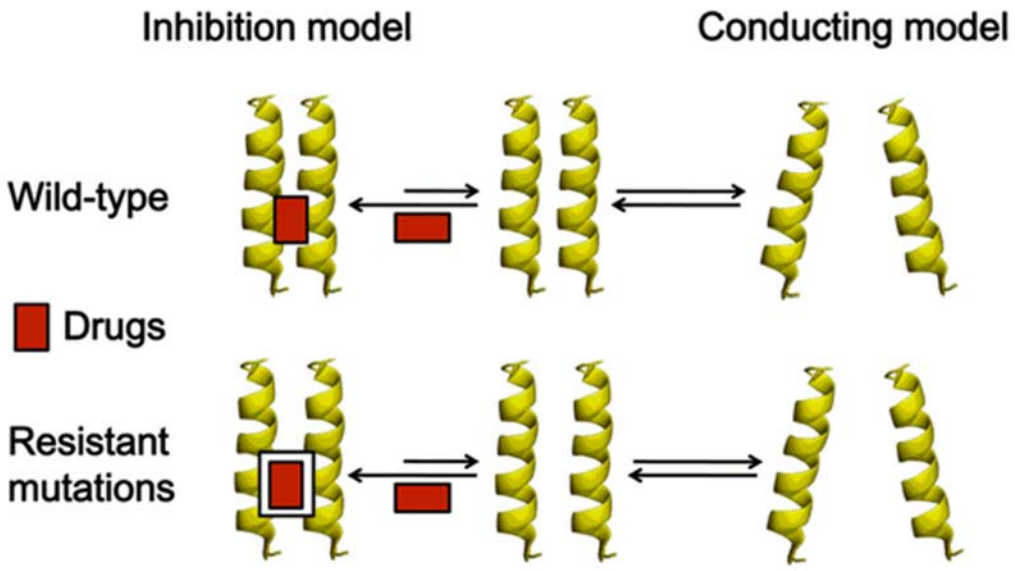

Fig. (4). High resolution structures of the M2 proton channel. (**A**) Solution NMR structure covering 43 residues (res.18-60) determined at pH 7.5 (2rlf.pdb). (**B**) Crystal structure covering 25 residues (res.22-46) determined in beta-octylglucoside detergent at pH 7.3 (3c9j.pdb).

position 31 (Ser31), and almost 10% occur at positions 27 (V27) and 30 (A30) [111]. Due to the different drug binding models (channel pore and lipid-facing binding modes), two mechanisms for the drug resistance are proposed. For the channel pore binding mode, it is reported that the drug resistance mainly comes from the different surrounding of the binding sites between wild-type and resistant mutation of the AM2 channel. In the wild-type AM2 channel, the suitable surrounding of the binding site can make a balance between hydrogen binding, hydrophobic interactions as well as the steric hindrance. However, in the resistant mutation of the AM2 channel, this surrounding is broken by mutations, making the interactions between two diagonal chains of the AM2 channel become stronger so as to result in a stronger steric hindrance. For the lipid-facing binding mode, an allosteric mechanism of the drug resistance is proposed [6,113]. As the mutations known to confer drug resistance are spread over 3 helical turns in the transmembrane domain [113], which cover an area much larger than the dimensions of the adamantane-based drugs, it is suggested that the drug resistance mechanism is more complicated than alteration of the surrounding of the channel pore binding site. According to the allosteric mechanism, the resistant mutations of the AM2 channel can weaken the packing interactions of the transmembrane helices so as to disrupt the lipid-facing binding pocket (Fig. **4**).

CONCLUSIONS AND PERSPECTIVES

A solid 3D structure of the M2 proton channel is not only the key for really understanding the life cycle of influenza viruses, but also indispensable for conducting rational drug design against the flu viruses. That is why for quite a long period of time in history, tremendous efforts have been made to determine the 3D structure of the M2 proton channel. Recently, the long-sough 3D structure of the M2 proton channel for influenza A [5,13] and that for influenza B [21] were consecutively successfully determined. These structures have provided a solid foundation, greatly stimulating various computational studies for in-depth understanding the subtle action mechanism of the M2 channel and rationally designing powerful drugs against influenza viruses that can overcome the drug-resistance problem.

This review is devoted to summarize the recent progresses in this regard. As we can see, the findings obtained via computational studies did timely provide useful insights into the detailed drug binding models at the atomic level, helping to reveal the subtle allosteric mechanism of the M2 channel during its drug-binding and proton-gating processes, as well as stimulating new strategy and design of new inhibitors to deal with the drug resistant problem against influenza viruses.

It is anticipated that further computational studies in this area should also take into account the effects of the global or "long-distance" interactions [114], as well as the protein internal motions since many marvelous biological functions are hardly really understood without considering these kinds of motions [115,116]. These kinds of internal motions are also vitally important to biomedicine [117,118]. Actually, investigation into the internal motion in biomacromolecules and its biological functions is deemed as a "genuinely new frontier in biological physics", as recently announced by the Vermont Company at its web site at http://homepages.sover.net/~bell/newFrontierpics.htm

ACKNOWLEDGEMENT

This work was supported by the grants from National Basic Research Program of China (973 Program, No.2010CB529205), National Natural Science Foundation of China (No.31000388 and 90913009), Program for New Century Excellent Talents in University (No.NCET-09-

551), Shanghai Natural Science Foundation (No.10ZR1421500), Shanghai "Phosphor" Science Foundation (No.10QA1403800), and Chinese Academy of Sciences (KSCX2-YW-R-112).

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

PATIENT CONSENT

Declared none.

REFERENCES

[1] Bouvier, N.M.; Palese, P. The biology of influenza viruses. *Vaccine*, **2008**, *26 Suppl 4*, D49-53.

[2] Pielak, R.M.; Chou, J.J. Flu channel drug resistance: a tale of two sites. *Protein Cell*, **2011**, *1*, 246-258.

[3] Cai, L.; Wang, Y.; Wang, J.F.; Chou, K.C. Identification of Proteins Interacting with Human SP110 During the Process of Viral Infections. *Med. Chem.*, **2011**, *7*, 121-126.

[4] Zebedee, S.L.; Lamb, R.A. Influenza A virus M2 protein: monoclonal antibody restriction of virus growth and detection of M2 in virions. *J. Virol.*, **1988**, *62*, 2762-2772.

[5] Schnell, J.R.; Chou, J.J. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature*, **2008**, *451*, 591-595.

[6] Pielak, R.M.; Chou, J.J. Influenza M2 proton channels. *Biochim. Biophys. Acta*, **2011**, *1808*, 522-529.

[7] Holsinger, L.J.; Lamb, R.A. Influenza virus M2 integral membrane protein is a homotetramer stabilized by formation of disulfide bonds. *Virology*, **1991**, *913*, 32-43.

[8] Sugune, R.J.; Hay, A.J. Structural characteristics of the M2 protein of influenza A virus: evidence that it forms a tetrameric channel. *Virology*, **1991**, *180*, 617-624.

[9] Chizhmakov, I.V.; Geraghty, F.M.; Ogden, D.C.; Hayhurst, A.; Antoniou, M.; Hay, A.J. Selective proton permeability and pH regulation of the influenza virus M2 channel expressed in mouse erythroleukaemia cells. *J. Physiol.*, **1996**, *494*, 329-336.

[10] Lakadamyali, M.; Rust, M.J.; Babcock, H.P.; Zhuang, X. Visualizing infection of individual influenza viruses. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 9280-9285.

[11] Huang, R.B.; Du, Q.S.; Wang, C.H.; Chou, K.C. An in-depth analysis of the biological functional studies based on the NMR M2 channel structure of influenza A virus. *Biochem. Biophys. Res. Comm.*, **2008**, *377*, 1243-1247.

[12] Hay, A.J.; Wolstenholme, A.J.; Skehel, J.J.; Smith, M.H. The molecular basis of the specific anti-influenza action of amantadine. *Embo. J.*, **1985**, *4*, 3021-3024.

[13] Stouffer, A.L.; Acharya, R.; Salom, D.; Levine, A.S.; Di Costanzo, L.; Soto, C.S.; Tereshko, V.; Nanda, V.; Stayrook, S.; DeGrado, W.F. Structural basis for the function and inhibition of an influenza virus proton channel. *Nature*, **2008**, *451*, 596-599.

[14] Chou, K.C.; Maggiora, G.M.; Nemethy, G.; Scheraga, H.A. Energetics of the structure of the four-alpha-helix bundle in proteins. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 4295-4299.

[15] Chou, K. C.; Maggiora, G.M.; Scheraga, H.A. The role of loop-helix interactions in stabilizing four-helix bundle proteins. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 7315-7319.

[16] Wei, H.; Wang, C. H.; Du, Q.S.; Meng, J.; Chou, K.C. Investigation into adamantane-based M2 inhibitors with FB-QSAR. *Med. Chem.*, **2009**, *5*, 305-317.

[17] Du, Q.S.; Huang, R.B.; Wang, S.Q.; Chou, K.C. Designing inhibitors of M2 proton channel against H1N1 swine influenza virus. *PLoS ONE*, **2010**, *5*, e9388.

[18] Du, Q.S.; Huang, R.B.; Wang, C.H.; Li, X.M.; Chou, K.C. Energetic analysis of the two controversial drug binding sites of the M2 proton channel in influenza A virus. *J. Theor. Biol.*, **2009**, *259*, 159-164.

[19] Salom, D.; Hill, B.R.; Lear, J.D.; DeGrado, W.F. pH-dependent tetramerization and amantadine binding of the transmembrane helix of M2 from the influenza A virus. *Biochemistry*, **2000**, *39*, 14160-14170.

[20] Tobler, K.; Kelly, M.L.; Pinto, L.H.; Lamb, R.A. Effect of cytoplasmic tail truncations on the activity of the M(2) ion channel of influenza A virus. *J. Virol.*, **1999**, *73*, 9695-9701.

[21] Wang, J.; Pielak, R.M.; McClintock, M.A.; Chou, J.J. Solution structure and functional analysis of the influenza B proton channel. *Nat. Struct. Mol. Biol.*, **2009**, *16*, 1267-1271.

[22] Pinto, L.H.; Dieckmann, G.R.; Gandhi, C.S.; Papworth, C.G.; Braman, J.; Shaughnessy, M.A.; Lear, J.D.; Lamb, R.A.; DeGrado, W.F. A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 11301-11306.

[23] Bauer, C.M.; Pinto, L.H.; Cross, T.A.; Lamb, R.A. The influenza virus M2 ion channel protein: probing the structure of the transmembrane domain in intact cells by using engineered disulfide cross-linking. *Virology*, **1999**, *254*, 196-209.

[24] Tian, C.; Gao, P.F.; Pinto, L.H.; Lamb, R.A.; Cross, T.A. Initial structural and dynamic characterization of the M2 protein transmembrane and amphipathic helices in lipid bilayers. *Protein Sci.*, **2003**, *12*, 2597-2605.

[25] Kukol, A.; Adams, P.D.; Rice, L.M.; Brunger, A.T.; Arkin, T.I. Experimentally based orientational refinement of membrane protein models: A structure for the Influenza A M2 H+ channel. *J. Mol. Biol.*, **1999**, *286*, 951-962.

[26] Kovacs, F.A.; Cross, T.A. Transmembrane four-helix bundle of influenza A M2 protein channel: structural implications from helix tilt and orientation. *Biophys. J.*, **1997**, *73*, 2511-2517.

[27] Song, Z.; Kovacs, F.A.; Wang, J.; Denny, J.K.; Shekar, S.C.; Quine, J.R.; Cross, T.A. Transmembrane domain of M2 protein from influenza A virus studied by solid-state (15)N polarization inversion spin exchange at magic angle NMR. *Biophys. J.*, **2000**, *79*, 767-775.

[28] Wang, J.; Kim, S.; Kovacs, F.; Cross, T.A. Structure of the transmembrane region of the M2 protein H(+) channel. *Protein Sci.*, **2001**, *10*, 2241-2250.

[29] Tian, C.; Tobler, K.; Lamb, R.A.; Pinto, L.H.; Cross, T.A. Expression and initial structural insights from solid-state NMR of the M2 proton channel from influenza A virus. *Biochemistry*, **2002**, *41*, 11294-11300.

[30] Hu, J.; Asbury, T.; Achuthan, S.; Li, C.; Bertram, R.; Quine, J.R.; Fu, R.; Cross, T.A. Backbone structure of the amantadine-blocked trans-membrane domain M2 proton channel from Influenza A virus. *Biophys. J.*, **2007**, *92*, 4335-4343.

[31] Chou, K.C. Energy-optimized structure of antifreeze protein and its binding mechanism. *J. Mol. Biol.*, **1992**, *223*, 509-517.

[32] Xie, Z.; Zhang, T.; Wang, J.F.; Chou, K.C.; Wei, D.Q. The computational model to predict accurately inhibitory activity for inhibitors towards CYP3A4. *Comput. Biol. Med.*, **2010**, *40*, 845-852.

[33] Chou, K.C. Insights from modelling the tertiary structure of BACE2. *J. Proteome Res.*, **2004**, *3*, 1069-1072.

[34] Chou, K.C. Insights from modelling the 3D structure of the extracellular domain of alpha7 nicotinic acetylcholine receptor. *Biochem. Biophys. Res. Commun.*, **2004**, *319*, 433-438.

[35] Wang, J.F.; Wei, D.Q.; Chen, C.; Li, Y.; Chou, K.C. Molecular modeling of two CYP2C19 SNPs and its implications for personalized drug design. *Protein Pept. Lett.*, **2008**, *15*, 27-32.

[36] Chou, K.C. Insights from modeling the 3D structure of DNA-CBF3b complex. *J. Proteome Res.*, **2005**, *4*, 1657-1660.

[37] Wang, J.F.; Wei, D.Q.; Chou, K.C. Drug candidates from traditional chinese medicines. *Curr. Top. Med. Chem.*, **2008**, *8*, 1656-1665.

[38] Chou, K.C. Modelling extracellular domains of GABA-A receptors: subtypes 1, 2, 3, and 5. *Biochem. Biophys. Res. Commun.*, **2004**, *316*, 636-642.

[39] Wang, J.F.; Wei, D.Q.; Li, L.; Zheng, S.Y.; Li, Y.X.; Chou, K.C. 3D structure modeling of cytochrome P450 2C19 and its implication for personalized drug design. *Biochem. Biophys. Res. Commun.*, **2007**, *355*, 513-519.

[40] Chou, K.C. Molecular therapeutic target for type-2 diabetes. *J. Proteome Res.*, **2004**, *3*, 1284-1288.

[41] Wang, J.F.; Wei, D.Q.; Lin, Y.; Wang, Y.H.; Du, H.L.; Li, Y.X.; Chou, K.C. Insights from modeling the 3D structure of NAD(P)H-dependent D-xylose reductase of Pichia stipitis and its binding interactions with NAD and NADP. *Biochem. Biophys. Res. Commun.*, **2007**, *359*, 323-329.

[42] Chou, K.C. Coupling interaction between thromboxane A2 receptor and alpha-13 subunit of guanine nucleotide-binding protein. *J. Proteome Res.*, **2005**, *4*, 1681-1686.

[43] Wang, J.F.; Chou, K.C. Insights from studying the mutation-induced allostery in the M2 proton channel by molecular dynamics. *Protein Eng. Des. Sel.*, **2010**, *23*, 663-666.

[44] Chou, K.C. Modeling the tertiary structure of human cathepsin-E. *Biochem. Biophys. Res. Commun.*, **2005**, *331*, 56-60.

[45] Yang, J. Molecular modeling of human BAD and its interaction with PKAc or PP1c. *J. Theor. Biol.*, **2009**, *257*, 159-169.

[46] Yang, J.; Li, J.H.; Wang, J.; Zhang, C.Y. Molecular modeling of BAD complex resided in a mitochondrion integrating glycolysis and apoptosis. *J. Theor. Biol*., **2010**, *266*, 231-241.

[47] Cheng, F.; Shen, J.; Xu, X.; Luo, X.; Chen, K.; Shen, X.; Jiang, H. Interaction models of a series of oxadiazole-substituted alpha-isopropoxy phenylpropanoic acids against PPARalpha and PPARgamma: molecular modeling and comparative molecular similarity indices analysis studies. *Protein Pept. Lett.*, **2009**, *16*, 150-162.

[48] Yang, J. Molecular modeling of human BAD, a pro-apoptotic Bcl-2 family member, integrating glycolysis and apoptosis. *Protein Pept. Lett.*, **2010**, *17*, 206-220.

[49] Zhang, R.; Wei, D.Q.; Du, Q.S.; Chou, K.C. Molecular modeling studies of peptide drug candidates against SARS. *Med. Chem.*, **2006**, *2*, 309-314.

[50] Wang, J.F.; Chou, K.C. Molecular modeling of cytochrome P450 and drug metabolism. *Curr Drug Metab*., **2010**, *11*, 342-346.

[51] Wang, J.F.; Wei, D.Q.; Chou, K.C. Insights from investigating the interactions of adamantane-based drugs with the M2 proton channel from the H1N1 swine virus. *Biochem. Biophys. Res. Commun*., **2009**, *388*, 413-417.

[52] Gu, R.X.; Gu, H.; Xie, Z.Y.; Wang, J.F.; Arias, H.R.; Wei, D.Q.; Chou, K.C. Possible drug candidates for Alzheimer's disease deduced from studying their binding interactions with alpha7 nicotinic acetylcholine receptor. *Med. Chem.*, **2009**, *5*, 250-262.

[53] Gong, K.; Li, L.; Wang, J.F.; Cheng, F.; Wei, D.Q.; Chou, K.C. Binding mechanism of H5N1 influenza virus neuraminidase with ligands and its implication for drug design. *Med. Chem.*, **2009**, *5*, 242-249.

[54] Chou, K.C.; Wei, D.Q.; Zhong, W.Z. Binding mechanism of coronavirus main proteinase with ligands and its implication to drug design against SARS. *Biochem. Biophys. Res. Commun.*, **2003**, *308*, 148-151.

[55] Liao, Q.H.; Gao, Q.Z.; Wei, J.; Chou, K.C. Docking and Molecular Dynamics Study on the Inhibitory Activity of Novel Inhibitors on Epidermal Growth Factor Receptor (EGFR). *Med. Chem.*, **2011**, *7*, 24-31.

[56] Xu, H. Inhibition Kinetics of Flavonoids on Yeast alpha-Glucosidase Merged with Docking Simulations. *Protein Pept. Lett.*, **2010**, *17*, 1270-1279.

[57] Wang, J.F.; Gong, K.; Wei, D.Q.; Li, Y.X.; Chou, K.C. Molecular dynamics studies on the interactions of PTP1B with inhibitors: from the first phosphate-binding site to the second one. *Protein Eng. Des. Sel*., **2009**, *22*, 349-355.

[58] Wang, J.F.; Chou, K.C. Insight into the molecular switch mechanism of human Rab5a from molecular dynamics simulations. *Biochem. Biophys. Res. Commun.*, **2009**, *390*, 608-612.

[59] Li, L.; Wei, D.Q.; Wang, J.F.; Chou, K.C. Computational studies of the binding mechanism of calmodulin with chrysin. *Biochem. Biophys. Res. Commun.*, **2007**, *358*, 1102-1107.

[60] Lian, P.; Wei, D.Q.; Wang, J.F.; Chou, K.C. An allosteric mechanism inferred from molecular dynamics simulations on phospholamban pentamer in lipid membranes. *PLoS ONE*, **2011**, *6*, e18587.

[61] Wang, J.F.; Chou, K.C. Insights from modeling the 3D structure of New Delhi metallo-β-lactamse and its binding interactions with antibiotic drug. *PLoS ONE*, **2011**, *6*, e18414.

[62] Wang, Y.; Wei, D.Q.; Wang, J.F. Molecular dynamics studies on T1 lipase: insight into a double-flap mechanism. *J. Chem. Inf. Model.*, **2010**, *50*, 875-878.

[63] Gu, H.; Chen, H.F.; Wei, D.Q.; Wang, J.F. Molecular dynamics simulations exploring drug resistance in HIV-1 proteases. *Chinese Sci. Bull.*, **2010**, *55*, 2677-2683.

[64] Wang, J.F.; Zhang, C.C.; Wei, D.Q. Docking and molecular dynamics studies on CYP2D6. *Chinese Sci. Bull.*, **2010**, *55*, 1877-1880.

[65] Chen, Q.; Zhang, T.; Wang, J.F.; Wei, D.Q. Advances in human cytochrome P450 and personalized medicine. *Curr. Drug Metab*. **2011**, *12*, 436-444.

[66] Du, Q.S.; Wang, S.Q.; Huang, R.B.; Chou, K.C. Computational 3D structures of drug-targeting proteins in the 2009-H1N1influenza A virus. *Chem. Phys. Lett.*, **2010**, *485*, 191-195.

[67] Sirois, S.; Wei, D.Q.; Du, Q.S.; Chou, K.C. Virtual Screening for SARS-CoV Protease Based on KZ7088 Pharmacophore Points. *J. Chem. Inf. Comput. Sci.*, **2004**, *44*, 1111-1122.

[68] Chou, K.C.; Wei, D.Q.; Du, Q.S.; Sirois, S.; Zhong, W.Z. Progress in computational approach to drug development against SARS. *Curr. Med. Chem.*, **2006**, *13*, 3263-3270.

[69] Chou, K. C.; Wei, D. Q.; Du, Q. S.; Sirois, S.; Shen, H. B.; Zhong, W. Z. Study of inhibitors against SARS coronavirus by computational approaches. In *Proteases in Biology and Disease: Viral proteases and antiviral protease inhibitor therapy* (Lendeckel, U. & Hooper, N. M., eds.), **2009**, Vol. *8*, pp. 1-23. Springer Science, Media B.V.

[70] Prado-Prado, F.J.; Martinez de la Vega, O.; Uriarte, E.; Ubeira, F.M.; Chou, K.C.; Gonzalez-Diaz, H. Unified QSAR approach to antimicrobials. 4. Multi-target QSAR modeling and comparative multi-distance study of the giant components of antiviral drug-drug complex networks. *Bioorg. Med. Chem.*, **2009**, *17*, 569-575.

[71] Dea-Ayuela, M.A.; Perez-Castillo, Y.; Meneses-Marcel, A.; Ubeira, F.M.; Bolas-Fernandez, F.; Chou, K.C.; Gonzalez-Diaz, H. HP-Lattice QSAR for dynein proteins: Experimental proteomics (2D-electrophoresis, mass spectrometry) and theoretic study of a Leishmania infantum sequence. *Bioorg. Med. Chem*., **2008**, *16*, 7770-7776.

[72] Du, Q.S.; Huang, R.B.; Chou, K.C. Recent advances in QSAR and their applications in predicting the activities of chemical molecules, peptides and proteins for drug design. *Curr. Protein Pept. Sci*., **2008**, *9*, 248-259.

[73] Du, Q.S.; Huang, R.B.; Wei, Y.T.; Du, L.Q.; Chou, K.C. Multiple Field Three Dimensional Quantitative Structure-Activity Relationship (MF-3D-QSAR). *J. Comput. Chem.*, **2008**, *29*, 211-219.

[74] Du, Q.S.; Huang, R.B.; Wei, Y.T.; Pang, Z.W.; Du, L.Q.; Chou, K.C. Fragment-Based Quantitative Structure-Activity Relationship (FB-QSAR) for Fragment-Based Drug Design. *J. Comput. Chem.*, **2009**, *30*, 295-304.

[75] Du, Q.S.; Mezey, P.G.; Chou, K.C. Heuristic Molecular Lipophilicity Potential (HMLP): A 2D-QSAR Study to LADH of Molecular Family Pyrazole and Derivatives. *J. Comput. Chem.*, **2005**, *26*, 461-470.

[76] Chou, K.C. Structural bioinformatics and its impact to biomedical science. *Curr. Med. Chem.*, **2004**, *11*, 2105-2134.

[77] Wang, J.F.; Wei, D.Q.; Chou, K.C. Pharmacogenomics and personalized use of drugs. *Curr. Top. Med. Chem.*, **2008**, *8*, 1573-1579.

[78] Lamb, R.A.; Zebedee, S.L.; Richardson, C.D. Influenza virus M2 protein is an integral membrane protein expressed on the infected-cell surface. *Cell*, **1985**, *40*, 627-633.

[79] Helenius, A. Unpacking the incoming influenza virus. *Cell*, **1992**, *69*, 577-578.

[80] Decoursey, T.E. Voltage-gated proton channels and other proton transfer pathways. *Physiol. Rev.*, **2003**, *83*, 475-579.

[81] Roux, B. Computational studies of the gramicidin channel. *Acc. Chem. Res.*, **2002**, *35*, 366-375.

[82] Chou, K.C.; Carlacci, L.; Maggiora, G.M.; Parodi, L.A.; Schultz, M.W. An energy-based approach to packing the 7-helix bundle of bacteriorhodopsin. *Protein Sci.*, **1992**, *1*, 810-827.

[83] Chou, K.C. Conformational change during photocycle of bacteriorhodopsin and its proton-pumping mechanism. *J. Protein Chem.*, **1993**, *12*, 337-350.

[84] Chou, K.C. A molecular piston mechanism of pumping protons by bacteriorhodopsin. *Amino Acids*, **1994**, *7*, 1-17.

[85] Pinto, L.H.; Lamb, R.A. The M2 proton channels of influenza A and B viruses. *J. Biol. Chem.*, **2006**, *281*, 8997-9000.

[86] Gandhi, C.S.; Shuck, K.; Lear, J.D.; Dieckmann, G.R.; DeGrado, W.F.; Lamb, R.A.; Pinto, L.H. Cu(II) inhibition of the proton

translocation machinery of the influenza A virus M2 protein. *J. Biol. Chem.*, **1999**, *274*, 5474-5482.

[87] Shuck, K.; Lamb, R.A.; Pinto, L.H. Analysis of the pore structure of the influenza A virus M(2) ion channel by the substituted-cysteine accessibility method. *J. Virol.*, **2000**, *74*, 7755-7761.

[88] Wang, C.; Lamb, R.A.; Pinto, L.H. Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. *Biophys. J.*, **1995**, *69*, 1363-1371.

[89] Pinto, L.H.; Holsinger, L.J.; Lamb, R.A. Influenza virus M2 protein has ion channel activity. *Cell*, **1992**, *69*, 517-528.

[90] Sansom, M.S.; Kerr, I.D.; Smith, G.R.; Son, H.S. The influenza A virus M2 channel: a molecular modeling and simulation study. *Virology*, **1997**, *233*, 163-173.

[91] Zhong, Q.; Husslein, T.; Moore, P.B.; Newns, D.M.; Pattnaik, P.; Klein, M.L. The M2 channel of influenza A virus: a molecular dynamics study. *FEBS Lett.*, **1998**, *434*, 265-271.

[92] Forrest, L.R.; Tieleman, D.P.; Sansom, M.S. Defining the transmembrane helix of M2 protein from influenza A by molecular dynamics simulations in a lipid bilayer. *Biophys. J.*, **1999**, *76*, 1886-1896.

[93] Forrest, L.R.; Kukol, A.; Arkin, I.T.; Tieleman, D.P.; Sansom, M.S. Exploring models of the influenza A M2 channel: MD simulations in a phospholipid bilayer. *Biophys. J.*, **2000**, *78*, 55-69.

[94] Schweighofer, K.J.; Pohorille, A. Computer simulation of ion channel gating: the M(2) channel of influenza A virus in a lipid bilayer. *Biophys. J.*, **2000**, *78*, 150-163.

[95] Zhong, Q.; Newns, D.M.; Pattnaik, P.; Lear, J.D.; Klein, M.L. Two possible conducting states of the influenza A virus M2 ion channel. *FEBS Lett.*, **2000**, *473*, 195-198.

[96] Smondyrev, A.M.; Voth, G.A. Molecular dynamics simulation of proton transport through the influenza A virus M2 channel. *Biophys. J.*, **2002**, *83*, 1987-1996.

[97] Wu, Y.; Voth, G.A. A computational study of the closed and open states of the influenza a M2 proton channel. *Biophys. J.*, **2005**, *89*, 2402-2411.

[98] Yi, M.; Cross, T.A.; Zhou, H.X. A secondary gate as a mechanism for inhibition of the M2 proton channel by amantadine. *J. Phys. Chem. B*, **2008**, *112*, 7977-7979.

[99] Yi, M.; Cross, T.A.; Zhou, H.X. Conformational heterogeneity of the M2 proton channel and a structural model for channel activation. *Proc. Natl. Acad. Sci. USA*, **2009**, *106*, 13311-13316.

[100] Mustafa, M.; Henderson, D.J.; Busath, D.D. Free-energy profiles for ions in the influenza M2-TMD channel. *Proteins*, **2009**, *76*, 794-807.

[101] Acharya, R.; Carnevale, V.; Fiorin, G.; Levine, B.G.; Polishchuk, A.L.; Balannik, V.; Samish, I.; Lamb, R.A.; Pinto, L.H.; DeGrado, W.F.; Klein, M.L. Structure and mechanism of proton transport through the transmembrane tetrameric M2 protein bundle of the influenza A virus. *Proc. Natl. Acad. Sci. USA*, **2010**, *107*, 15075-15080.

[102] Balannik, V.; Carnevale, V.; Fiorin, G.; Levine, B.G.; Lamb, R.A.; Klein, M.L.; Degrado, W.F.; Pinto, L.H. Functional studies and modeling of pore-lining residue mutants of the influenza a virus M2 ion channel. *Biochemistry*, **2010**, *49*, 696-708.

[103] Manor, J.; Mukherjee, P.; Lin, Y.S.; Leonov, H.; Skinner, J.L.; Zanni, M.T.; Arkin, I.T. Gating mechanism of the influenza A M2 channel revealed by 1D and 2D IR spectroscopies. *Structure*, **2009**, *17*, 247-254.

[104] Pielak, R.M.; Chou, J.J. Kinetic analysis of the M2 proton conduction of the influenza virus. *J. Am. Chem. Soc.*, **2010**, *132*, 17695-17697.

[104] Khurana, E.; Dal Peraro, M.; DeVane, R.; Vemparala, S.; DeGrado, W.F.; Klein, M.L. Molecular dynamics calculations suggest a conduction mechanism for the M2 proton channel from influenza A virus. *Proc. Natl. Acad. Sci. USA*, **2009**, *106*, 1069-1074.

[106] Carnevale, V.; Fiorin, G.; Levine, B.G.; Degrado, W.F.; Klein, M.L. Multiple Proton Confinement in the M2 Channel from the Influenza A Virus. *J. Phys. Chem. C*, **2010**, *114*, 20856-20863.

[107] Chen, H.; Wu, Y.; Voth, G.A. Proton transport behavior through the influenza A M2 channel: insights from molecular simulation. *Biophys. J.*, **2007**, *93*, 3470-3479.

[108] Phongphanphanee, S.; Rungrotmongkol, T.; Yoshida, N.; Hannongbua, S.; Hirata, F. Proton transport through the influenza A M2 channel: three-dimensional reference interaction site model study. *J. Am. Chem. Soc.*, **2010**, *132*, 9782-9788.

[109] Intharathep, P.; Laohpongspaisan, C.; Rungrotmongkol, T.; Loisruangsin, A.; Malaisree, M.; Decha, P.; Aruksakunwong, O.; Chuenpennit, K.; Kaiyawet, N.; Sompornpisut, P.; Pianwanit, S.; Hannongbua, S. How amantadine and rimantadine inhibit proton transport in the M2 protein channel. *J. Mol. Graph. Model.*, **2008**, *27*, 342-348.

[110] Zarubaev, V.V.; Golod, E.L.; Anfimov, P.M.; Shtro, A.A.; Saraev, V.V.; Gavrilov, A.S.; Logvinov, A.V.; Kiselev, O.I. Synthesis and anti-viral activity of azolo-adamantanes against influenza A virus. *Bioorg. Med. Chem.*, **2009**, *18*, 839-848.

[111] Suzuki, H.; Saito, R.; Masuda, H.; Oshitani, H.; Sato, M.; Sato, I. Emergence of amantadine-resistant influenza A viruses: epidemiological study. *J. Infect. Chemother*, **2003**, *9*, 195-200.

[112] Cady, S.D.; Schmidt-Rohr, K.; Wang, J.; Soto, C.S.; Degrado, W.F.; Hong, M. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature*, **2010**, *463*, 689-692.

[113] Pielak, R.M.; Schnell, J.R.; Chou, J.J. Mechanism of drug inhibition and drug resistance of influenza A M2 channel. *Proc. Natl. Acad. Sci. USA*, **2009**, *106*, 7379-7384.

[114] Chou, K.C. Prediction of tight turns and their types in proteins. *Anal. Biochem.*, **2000**, *286*, 1-16.

[115] Chou, K.C. Low-frequency collective motion in biomacromolecules and its biological functions. *Biophys. Chem.*, **1988**, *30*, 3-48.

[116] Chou, K.C. Low-frequency resonance and cooperativity of hemoglobin. *Trends Biochem. Sci.*, **1989**, *14*, 212.

[117] Gordon, G. Extrinsic electromagnetic fields, low frequency (phonon) vibrations, and control of cell function: a non-linear resonance system. *J. Biomed. Sci. Eng.*, **2008**, *1*, 152-156.

[118] Madkan, A.; Blank, M.; Elson, E.; Chou, K.C.; Geddis, M.S.; Goodman, R. Steps to the clinic with ELF EMF. *Nat. Sci.*, **2009**, *1*, 157-165.

Received: April 12, 2011 Revised: May 05, 2011 Accepted: May 07, 2011