The Functional Domain Analysis of TLR9 with the Visualization of the Energetically Allowed Region

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The Functional Domain Analysis of TLR9 with the Visualization of the Energetically Allowed Region

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Abstract. Toll-Like Receptor 9 (TLR9) is one of the members of the toll-like receptor family that plays a pivotal role in sensing pathogen-associated molecular patterns that are harmful to human body. Upon activation, these molecular patterns trigger the downstream signaling pathways which eventually leading to immune defense. This study aims at using big data approach in exploring the functional domains of TLR9. The acquired results and analyses provide an insight for future studies in the domain of immunosurveillance.

1. Introduction

Toll-like receptors (TLRs) are a class of protein that features in the innate recognition of a wide variety of pathogens and danger signals, by detecting a specified range of pathogen-associated or damage-associated molecular patterns [1-3]. Cell-surface TLRs such as TLR4 and TLR1/2 are capable of detecting extracellular microbes; whereas intracellular TLRs such as TLR3 and TLR9 are capable of recognizing microbial nucleic acids [4]. TLRs are the most characterized pattern recognition receptors that consist of three types of domains, which are the extracellular ectodomains containing leucine-rich repeats, a trans-membrane domain, and an intracellular toll-interleukin-1 receptor domain [5]. Aberrant expression of TLRs and the malfunctioning of TLR signaling pathways are implicated in various diseases, such as autoimmune diseases [6], infectious diseases [7], inflammatory diseases [8], tumorigenesis [9], and motor abnormalities [10].

TLR9 as a member of the TLRs family is a receptor for unmethylated CpG DNA [11]. It is expressed by various immune cells such as CD4+ T cells [12] and dendritic cells [13]. TLR9 ligands are single-stranded DNAs (ssDNAs) characterized with unmethylated CpG motif, which is prevalent in microorganisms [11]. Research has shown that TLR7/TLR9 signaling pathways can induce the production of type I interferons and proinflammatory cytokines [13]. The expression of TLR7 and TLR9 in B cells plays an important role in the regulation of an array of function in B cells. It was found that the balance between TLR7 and TLR9 molecules in B cells is critical in the development of B cell autoreactivity, which has profound implications in the immunity [14]. In the context of TLR7/TLR9 and B cell receptor signaling crosstalk, the degree of TLR9 responsiveness is dependent on the alterations of certain types of B-cell signaling molecules and the cellular milieu [14].

In response to viral ssDNAs in plasmacytoid dendritic cells (pDCs), TLR9 recruits MyD88, which is an adaptor protein that forms an aggregate with Interleukin-1 receptor-associated kinase 1 (IRAK-1), Interleukin-1 receptor-associated kinase 4 (IRAK-4), and Interferon regulatory factor 7 (IRF-7), all of which playing the role in transcriptional activation [15]. Phosphorylated IRF-7 translocates into the
nucleus to activate the promoters of type I interferon, a type of signaling protein that is implicated in the innate immunologic defense against viral infections [16].

Despite the progress in experimental studies, large amount of data produced in the laboratory requires various computational techniques and strategies for analysis and prediction. Various computational approaches have been adopted to analyze the enormous amount of molecular data [17]. In the recent years, computational models have been built to determine the interaction of the TLR9 with CpG oligodeoxynucleotides [18]. Interaction between bacteria and toll-like receptor mediated inflammation has been modeled mathematically in the necrotizing enterocolitis context [19]. The model predicts that TLR-9 can inhibit both the beneficial and harmful effects of TLR4, a finding suggests that a synergistic balance of action between TLR4 and TLR9 is indispensable in maintaining intestinal homeostasis [19]. In this study, we strive to enhance the functional domain analysis of TLR9 using a range of computational approaches such as web services and big data analysis tools.

2. Methods
We used Protein Data Bank [20] to identify protein structural information of TLR9. A neural-network-based Peons public web server [21] was used to predict the best matched template for TLR9. Ramachandran plot [22] and the relevant techniques in the domain of big data was used to display $\Psi$ (backbone dihedral angles) against $\phi$ (amino acid residual sequences). We contend that the backbone of a protein to be interpreted as a class of discrete curves that permits the Frenet frames to be calculated [23]. Let

$$S_j = |P_{j+1} - P_j|$$  \hspace{1cm} (1)

a unit tangent vector at point $P_j$, where $j=0, \ldots, n-1$, is given as such:

$$t_j = \frac{P_{j+1} - P_j}{S_j}$$  \hspace{1cm} (2)

$$P_k - P_0 = \sum_{j=0}^{k-1} S_j t_j$$  \hspace{1cm} (3)

3. Results and Discussion
We used a mice crystal structure of TLR9 in the complex with the inhibitory DNA4084 (form1) as our PDB input file (PDB ID: 3WPG), as obtained from NGL viewer [24]. Figure 1 demonstrates a 3D view of 3WPG.

![Figure 1: 3D view of 3WPG](image-url)
We used the Ramachandran plot to probe the energetically permitted regions of the residues of 3WPG. We used the server hosted in the Cambridge University to perform the analysis. The plot in the figure shows each amino acid residue as a dot area in a diagram of $\phi$ against $\Psi$. The amino acid residues in a favored region and generously allowed regions are displayed in small red and yellow dot, respectively, in Figure 2 and Figure 3 below. We used the structure validation method developed by Lovell et al. [25].

![Ramachandran Plot for 3WPG](image)

**Figure 2: The 1st Ramachandran Plot for 3WPG**
In our analysis of the residues of 3WPG, 50 residues (7%) are in the allowed region. Besides, it was found that 664 residues (93%) are in the favored region of the plot. None was found in the outlier region. Due to the limit of space, only partial analyzed results are shown in Figure 4.
4. Conclusion

TLR9 is a critical pathogen pattern detection protein in human body. A complete insight of its structural, biochemical, and molecular features is vital in molecular medicine and applied research. In this paper, the functional domains of TLR9 was probed using computational tools coupled with the big data approach. Residue properties were analyzed to validate the structure of 3WPG, which is a crystal structure of TLR9 in mouse models. Ramachandran plots show that most of the amino acid residues in our study emerge in the most favored regions. The molecular characteristics of 3WPG thus provide insights into the functional domains of TLR9.

References

[1] S. Akashi-Takamura and K. Miyake, “TLR accessory molecules,” Current Opinion in Immunology, vol. 20, pp. 420-425, 2008.
[2] G. Trinchieri and A. Sher, “Cooperation of toll-like receptor signals in innate immune defence,” Nature Reviews Immunology, vol. 7, pp. 179-190, 2007.
[3] T. Kawai and S. Akira, “The role of pattern-recognition receptors in innate immunity: update on toll-like receptors,” Nature Immunology, vol. 11, pp. 373-384, 2010.
[4] T. Kaisho and S. Akira, “Toll-like receptor function and signaling,” Journal of Allergy and Clinical Immunology, vol. 117, pp. 979-990, 2006.
[5] T. Kondo, T. Kawai, and S. Akira, “Dissecting negative regulation of toll-like receptor signaling,” Trends in Immunology, vol. 33, pp. 449-458, 2012.
[6] M. Ehlers and J. Ravetch, “Opposing effects of Toll-like receptor stimulation induce autoimmunity or tolerance,” Trends in Immunology, vol. 28, pp. 74-79, 2007.
[7] V. Balloy, M. Si-Tahar, O. Takeuchi, B. Philippe, M-A. Nahori, M. Tanguy, M. Huerre, S. Akira, J-P. Latgé, and M. Chignard, “Involvement of Toll-like receptor 2 in experimental invasive pulmonary aspergillosis,” Infection and Immunity, vol. 73, pp. 979-989, 2005.
[8] K.H. Shalaby, S. Al Heily, K. Tsuchiy, S. Farahm, T.McGovern, P.A. Risse, W.K. Suh, S.T. Qureshi, and J.G. Martin, “The TLR4-TRIF pathway can protect against the development of experimental allergic asthma,” Immunology, 2017, doi:10.1111/imm.12755
[9] I. Hirsch, C. Caux, U. Hasan, N. Bendriss-Vermare, and D. Olive, “Impaired Toll-like receptor 7 and 9 signaling: from chronic viral infections to cancer,” Trends in Immunology, vol. 31, pp. 391-397, 2010.

[10] V. Patel, A.M. Patel, and J.J. McArdle, “Synaptic abnormalities of mice lacking toll-like receptor (TLR)-9,” Neuroscience, vol. 324, pp. 1-10, 2016.

[11] H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira, “A Toll-like receptor recognizes bacterial DNA,” Nature, vol. 408, pp. 740-745, 2000.

[12] J. Reynolds and C. Dong, “Toll-like receptor regulation of effector T lymphocyte function,” Trends in Immunology, vol. 34, pp. 511-519, 2013.

[13] K. Hoshino and T. Kaisho, “Nucleic acid sensing Toll-like receptors in dendritic cells,” Current Opinion in Immunology, vol. 20, pp. 408-413, 2008.

[14] A.N. Suthers and S. Sarantopoulos, “TLR7/TLR9- and B cell receptor-signaling crosstalk: Promotion of potentially dangerous B cells,” Frontiers in Immunology, 8:775, doi:10.3389/fimmu.2017.00775, 2017.

[15] T. Kawai, S. Sato, K.J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. Inoue, S. Uematsu, O. Takeuchi, and S. Akira, “Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6,” vol. 5(10), pp. 1061-1068, 2004.

[16] G.A. Versteeg and A. García-Sastre, “viral tricks to grid-lock the type I interferon system,” Current Opinion in Microbiology, vol. 13, pp. 508-516, 2010.

[17] J. Konc and D. Janežič, “ProBiS-2012: web server and web services for detection of structurally similar binding sites in proteins,” Nucleic Acids Research, vol. 40, pp. W214-W221, 2012.

[18] W. Zhou, Y. Li, X. Pan, Y. Gao, B. Li, Z. Qiu, L. Liang, H. Zhou, and J. Yue, “Toll-like receptor 9 interaction with CpG ODN: An in silico analysis approach,” Theoretical Biology and Medical Modelling, 10:18, 2013.

[19] J. Arciero, E.G. Bard, R. Siggers, A. Afradi, D. Hackam, Y. Vodovotz, and J. Rubin, “Modeling the interactions of bacteria and Toll-like receptor-mediated inflammation in necrotizing enterocolitis,” Journal of Theoretical Biology, vol. 321, pp. 83-99, 2013.

[20] H. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, H. Weissig, I. Shindyalov, and P. Bourne, “The Protein Data Bank,” Nucleic Acids Research, vol. 28, pp. 235-242, 2000.

[21] J. Lundstrom, L. Rychlewski, J. Buńnicki, and A. Elofsson, “Pcons: a neural-network-based consensus predictor that improves fold recognition,” Protein Science, vol. 10, pp. 2354-2362, 2001.

[22] G. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, “Stereochemistry of polypeptide chain configurations,” Journal of Molecular Biology, vol. 7, pp. 95-99, 1963.

[23] J. Quine, T. Cross, M. Chapman, and R. Bertram, “Mathematical aspects of protein structure determination with NMR orientational restraints,” Bulletin of Mathematical Biology, vol. 66, pp. 1705-1730, 2004.

[24] A.S. Rose, A.R. Bradley, Y. Valsalata, J.M. Duarte, A. Pričić, and P.W. Rose, “Web-based molecular graphics for large complexes,” ACM Proceedings of the 21st International Conference on Web 3D Technology (Web3D ’16), pp. 185-186, 2016.

[25] S.C. Lovell, J.W. Davis, W. Arendall, P. de Bakker, J. Word, M.G. Prisant, J. Richardson, D.C. Richardson, “Structure validation by Calpha geometry: phi, psi and Cbeta deviation,” Proteins, vol 50(3), pp. 437-450, 2003.