Druggability Analysis of Membrane Proteins by DoGSiteScorer

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ABSTRACT: Membrane proteins are the most medicinally important yet to be fully exploited pharmaceutical targets. Here druggability analyses are conducted on three different membrane proteins, namely, the human P2Y12 receptor, glycoprotein-41 that mediates the HIV-1 virus entry and membrane fusion, and phospholamban that regulates the Ca^{2+} pump in cardiac muscle cells. DoGSiteScorer, a grid-based bioinfromatic technology, is able to identify the binding pockets of all three membrane proteins, and the results were in great agreements with the available crystal structure of P2Y12 receptor-ligand complex. This druggability analysis is especially helpful in cases where the crystal structures of membrane protein-ligand complexes are still difficult to obtain. Better understanding of the druggable pockets of membrane proteins also requires including the membrane environment.

Key Words: druggability, membrane protein, HIV, glycoprotein-41, phospholamban, DoGSiteScorer

INTRODUCTION:

Membrane proteins are proteins that interact with or are part of biological membranes and it is estimated that membrane proteins represent over 40% of pharmaceutical targets(Overington, Al-Lazikani, & Hopkins, 2006). Most structure-based drug design relays heavily on understanding the pockets, cavities, or grooves on the surface of protein targets. Identification, comparison and characterization of the potential binding sites (pockets, cavities, or grooves) are pivotal to structure-based drug design endeavors.

Recently, the number of crystal structures of membrane proteins in the Protein Data Bank (PDB) has been increasing considerably due to the development of new technologies (David C. Chan, Chutkowski, & Kim, 1998; Irimia, Sarkar, Stanfield, & Wilson, 2016; Rosenbaum, Rasmussen, & Kobilka, 2009). In addition to crystallography, NMR spectroscopy has been a very powerful technology especially to investigate the

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interactions of drug (drug-like) small molecules, proteins and lipid membranes (Cady et al., 2010; S. Chu, Hawes, & Lorigan, 2009; S. Chu, Maltsev, Emwas, & Lorigan, 2010; Schnell & Chou, 2008). Very recently, cryo-EM is providing more structural information especially on huge membrane protein complexes (Byeon et al., 2009; Lee, Ozorowski, & Ward, 2016; X. Zhang, Jin, Fang, Hui, & Zhou, 2010).

On the other hand, structural bioinformatics is another important approach and component to the structural biology (Kuo-Chen, 2004). Many bioinformatic tools have been developed to assist our understanding of how small molecule drugs and macromolecules work (Pérot, Sperandio, Miteva, Camproux, & Villoutreix, 2010). DoGSiteScorer is a grid-based method which uses a Difference of Gaussian filter to detect potential binding pockets - solely based on the 3D structure of the protein (A. Volkamer, Kuhn, Rippmann, & Rarey, 2012). Several global properties, describing the size, shape, depth, biophysical and chemical features of the predicted pockets are calculated. Per default, a simple druggability score (0–1) is calculated for each (sub) pocket, and the higher the score the more druggable the pocket is estimated to be. DoGSiteScorer has been used more and more in various applications in analyzing the druggability of pharmaceutical targets including a few membrane proteins(Aretz, Wamhoff, Hanske, Heymann, & Rademacher, 2014; Lima et al., 2016; Andrea Volkamer et al., 2015; Andrea Volkamer, Kuhn, Grombacher, Rippmann, & Rarey, 2012).

In this paper we investigated the druggability of three membrane proteins: human P2Y12 receptor, glycoprotein-41 and phospholamban. G-protein-coupled receptors (GPCRs) are the largest (account for 12% of all human protein targets) and most privileged groups of membrane receptors(Santos et al., 2017). Here we choice a P2Y receptors (P2YRs) (K. Zhang et al., 2014), a family of purinergic GPCRs as a reprehensive target to conduct druggability analysis using DoGSiteScorer. HIV glycoprotein 41 (gp41) represents an interesting membrane protein target for potential protein-protein interaction modulator. Gp41 is coiled-coil.(D. C. Chan, Fass, Berger, & Kim, 1997) This represented a potential pharmaceutical target for entry inhibitors(Zhou & Chu, 2013). Several gp41 have been prepared for drug discovery (S. D. Chu et al., 2015; Walsh, Chu, Zhang, & Gochin, 2015). Chu et al have discovered another binding site on gp41 by using a novel fragment library screening combined with chemoinformatics (S. D. Chu & Gochin, 2013). Phospholamban is a membrane protein(MacLennan & Kranias, 2003). Recently several important studies have been published on the structure of PLB in biological membrane environments that provided more insights on how this membrane protein work as an key player in regulating Ca^{2+} transfer across the cell membrane that relating to heart diseases (S. Chu, Abu-Baker, Lu, & Lorigan, 2010; S. Chu, Coey, & Lorigan, 2010; SIMMERMAN & JONES, 1998). Full understandings of the possible binding pocket of these proteins are still required especially to develop specific and effective small molecules agents to interfering these important membrane proteins in disease-related backgrounds.

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METHODS

All druggability analysis were conducted by using DoGSiteScorer (A. Volkamer et al., 2012) server ((http://dogsite.zbh.uni-hamburg.de). The PDB codes used in druggability analysis were **4NTJ** for the P2Y12 receptor **(K. Zhang et al., 2014)**, **1AIK** for HIV-1 glycoprotein-41 (gp41) (D. C. Chan et al., 1997), **1FJK** and **1FJP** for Phospholamban (PLB) **(Lamberth et al., 2000)**, respectively.

RESULTS AND DISCUSSIONS

Druggability Analysis of the Human P2Y12 Receptor

Fist, the druggability of the human P2Y12 receptor (PDB code: **4NTJ**) (K. Zhang et al., 2014) was analyzed by DoGSiteScorer to see if this bioinformatic tool can identify the binding site of this membrane protein, the PDB structure of which shows the binding site experimentally.

In **Figure 1**, the top two pockets identified by DoGSiteScorer were demonstrated and the major indexes describing the pockets were summarized. DoGSiteScorer successfully identified the pocket (P_1, colored in purple) where the lignad AZJ_A_1201 bind to in the crystal structure **4NTJ**, as demonstrated by overlaying the predicted binding pocket and the ligand AZJ_A_1201 in the structure picture in the middle of **Figure 1**. This analysis result, along with other published literature (Li et al., 2014), confirmed the capability of DoGSiteScorer in identifying the binding pockets of membrane proteins.

Druggability Analysis of HIV Glycoprotein-41

After evaluating the performance of DoGSiteScorer by using a PDB structure with an already known binding site, the human P2Y12 receptor, the druggability of HIV-1 glycoprotein (gp41) was analyzed by using the same bioinformatic technology.

Until know, it has been proved very difficult to obtain a crustal structure of HIV-1 gp41 with a binding small molecule Ligand, although small molecule HIV entry inhibitors have been goals of several very active researches groups(Zhou & Chu, 2013) . Experimentally, NMR spectroscopy has been used successfully to indentify the two binding sites of gp41 (S. D. Chu & Gochin, 2013; S. D. Chu et al., 2015).

In **Figure 2**, three pockets of gp41 were identified by using the gp41 crustal structure PDB code **1AIK** (David C. Chan et al., 1998) in DoGSiteScorer druggability analysis. Pocket_0 is located in the loop region that connecting NHR and CHR helixes. Pocket_1 and Pocket_2 are located on the surface of the helix surface and are in good agreements with the experimental data, confirming the binding pockets of gp41 determined by NMR spectroscopy (S. D. Chu & Gochin, 2013; S. D. Chu et al., 2015). These two binding pockets provide very valuable targets for developing small molecule inhibitors that can inhibit the HIV virus entering into the host cells.

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Druggability Analysis of Phospholamban

Finally the druggability of phospholamban (PLB) was analyzed by using two PDB structures: **1FJK** (the PLB structure without membrane), and **FJP** (the PLB structure in detergent micelles). The micelles are mimics of biological lipid membrane environment.

As shown in **Figure 3**, very interestingly, only one druggable pocket was detected by DoGSiteScorer in the PLB structure in the membrane (**1FJP**), while two pockets were detected in the PLB structure without membrane (**1FJK**).

The membrane environment seems making the Pocket_1 (pink colored) of PLB inaccessible. This is in good agreements with the NMR determined topology and structures of PLB in lipid membranes (S. Chu, Abu-Baker, et al., 2010; S. Chu, Coey, et al., 2010). This highlights the critical roles of membrane environments, which should be included in druggability analysis of membrane proteins. Researchers at Orion Pharma and University of Helsinki have published small molecule inhibitors of PLB by using a phage display library to identify cyclic peptides with bidding affinity for a soluble portion of PLB, and then developed small molecules that mimic these cyclic peptides as Phospholamban inhibitors(Tilgmann et al., 2013). The binding amino acids in that complex confirmed that Arg9 13 and 14 on PLB were participated to the binding of PLB to the inhibitors.

CONCLUSIONS

Druggability analysis of membrane proteins was investigated by using three different proteins: human P2Y12 receptor, HIV-1 glycoprotein-41 and phospholamban. The top binding pockets of human P2Y12 receptor identified by DoGSiteScorer were in great agreement with the available crystal structure of the protein-ligand complex. For membrane proteins that the crystal structures of protein-ligand complexes are very difficult to obtain, such as HIV-1 glycoprotein-41 (gp41), DoGSiteScorer successfully predicted the two binding pockets that have been confirmed by NMR spectroscopy studies. The effect of membrane environment was also successfully evaluated by using Phospholamban (PLB) as an example. This study demonstrated that druggability analysis by using bioinformatic tools such as DoGSiteScorer is very helpful in drug discovery endeavors targeting at disease-related membrane proteins and the membrane environment should be taken into consideration.

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REFERENCES

Aretz, J., Wamhoff, E.-C., Hanske, J., Heymann, D., & Rademacher, C. (2014). Computational and Experimental Prediction of Human C-Type Lectin Receptor Druggability. *Frontiers in Immunology, 5*(323). doi: 10.3389/fimmu.2014.00323

Byeon, I.-J. L., Meng, X., Jung, J., Zhao, G., Yang, R., Ahn, J., . . . Gronenborn, A. M. (2009). Structural Convergence between Cryo-EM and NMR Reveals Intersubunit Interactions Critical for HIV-1 Capsid Function. *Cell, 139*(4), 780-790. doi: 10.1016/j.cell.2009.10.010

Cady, S. D., Schmidt-Rohr, K., Wang, J., Soto, C. S., DeGrado, W. F., & Hong, M. (2010). Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature, 463*(7281), 689-692. doi: http://www.nature.com/nature/journal/v463/n7281/suppinfo/nature08722_S1.html

Chan, D. C., Chutkowski, C. T., & Kim, P. S. (1998). Evidence that a prominent cavity in the coiled coil of HIV type 1 gp41 is an attractive drug target. *Proceedings of the National Academy of Sciences of the United States of America, 95*(26), 15613-15617.

Chan, D. C., Fass, D., Berger, J. M., & Kim, P. S. (1997). Core structure of gp41 from the HIV envelope glycoprotein. *Cell, 89*(2), 263-273. doi: 10.1016/s0092-8674(00)80205-6

Chu, S., Abu-Baker, S., Lu, J., & Lorigan, G. A. (2010). 15N Solid-state NMR spectroscopic studies on phospholamban at its phosphorylated form at Ser-16 in aligned phospholipid bilayers. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1798*(3), 312-317. doi: http://dx.doi.org/10.1016/j.bbamem.2009.12.020

Chu, S., Coey, A. T., & Lorigan, G. A. (2010). Solid-state 2H and 15N NMR studies of side-chain and backbone dynamics of phospholamban in lipid bilayers: Investigation of the N27A mutation. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1798*(2), 210-215. doi: http://dx.doi.org/10.1016/j.bbamem.2009.09.025

Chu, S., Hawes, J. W., & Lorigan, G. A. (2009). Solid-state NMR spectroscopic studies on the interaction of sorbic acid with phospholipid membranes at different pH levels. *Magnetic Resonance in Chemistry, 47*(8), 651-657. doi: 10.1002/mrc.2444

Chu, S., Maltsev, S., Emwas, A. H., & Lorigan, G. A. (2010). Solid-state NMR paramagnetic relaxation enhancement immersion depth studies in phospholipid bilayers. *Journal of Magnetic Resonance, 207*(1), 89-94. doi: http://dx.doi.org/10.1016/j.jmr.2010.08.012

Chu, S. D., & Gochin, M. (2013). Identification of fragments targeting an alternative pocket on HIV-1 gp41 by NMR screening and similarity searching. *Bioorganic & Medicinal Chemistry Letters, 23*(18), 5114-5118. doi: 10.1016/j.bmcl.2013.07.026

Chu, S. D., Kaur, H., Nemati, A., Walsh, J. D., Partida, V., Zhang, S. Q., & Gochin, M. (2015). Swapped-Domain Constructs of the Glycoprotein-41 Ectodomain Are Potent Inhibitors of HIV Infection. *Acs Chemical Biology, 10*(5), 1247-1257. doi: 10.1021/cb501021j

Irimia, A., Sarkar, A., Stanfield, Robyn L., & Wilson, Ian A. (2016). Crystallographic Identification of Lipid as an Integral Component of the Epitope of HIV Broadly Neutralizing Antibody 4E10. *Immunity, 44*(1), 21-31. doi: 10.1016/j.immuni.2015.12.001

Kuo-Chen, C. (2004). Structural Bioinformatics and its Impact to Biomedical Science. *Current Medicinal Chemistry, 11*(16), 2105-2134. doi: http://dx.doi.org/10.2174/0929867043364667

Lamberth, S., Schmid, H., Muenchbach, M., Vorherr, T., Krebs, J., Carafoli, E., & Griesinger, C. (2000). NMR Solution Structure of Phospholamban. *Helvetica Chimica Acta, 83*(9), 2141-2152. doi: 10.1002/1522-2675(20000906)83:9<2141::AID-HLCA2141>3.0.CO;2-W

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Lee, J. H., Ozorowski, G., & Ward, A. B. (2016). Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. *Science, 351*(6277), 1043-1048. doi: 10.1126/science.aad2450

Li, Y., To, J., Verdià-Baguena, C., Dossena, S., Surya, W., Huang, M., . . . Torres, J. (2014). Inhibition of the Human Respiratory Syncytial Virus Small Hydrophobic Protein and Structural Variations in a Bicelle Environment. *Journal of Virology, 88*(20), 11899-11914. doi: 10.1128/JVI.00839-14

Lima, A. N., Philot, E. A., Trossini, G. H. G., Scott, L. P. B., Maltarollo, V. G., & Honorio, K. M. (2016). Use of machine learning approaches for novel drug discovery. *Expert Opinion on Drug Discovery, 11*(3), 225-239. doi: 10.1517/17460441.2016.1146250

MacLennan, D. H., & Kranias, E. G. (2003). Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol, 4*(7), 566-577.

Overington, J. P., Al-Lazikani, B., & Hopkins, A. L. (2006). How many drug targets are there? *Nat Rev Drug Discov, 5*(12), 993-996.

Pérot, S., Sperandio, O., Miteva, M. A., Camproux, A.-C., & Villoutreix, B. O. (2010). Druggable pockets and binding site centric chemical space: a paradigm shift in drug discovery. *Drug Discovery Today, 15*(15–16), 656-667. doi: http://dx.doi.org/10.1016/j.drudis.2010.05.015

Rosenbaum, D. M., Rasmussen, S. G. F., & Kobilka, B. K. (2009). The structure and function of G-protein-coupled receptors. *Nature, 459*(7245), 356-363.

Santos, R., Ursu, O., Gaulton, A., Bento, A. P., Donadi, R. S., Bologa, C. G., . . . Overington, J. P. (2017). A comprehensive map of molecular drug targets. *Nat Rev Drug Discov, 16*(1), 19-34. doi: 10.1038/nrd.2016.230

http://www.nature.com/nrd/journal/v16/n1/abs/nrd.2016.230.html#supplementary-information

Schnell, J. R., & Chou, J. J. (2008). Structure and mechanism of the M2 proton channel of influenza A virus. *Nature, 451*(7178), 591-595. doi: http://www.nature.com/nature/journal/v451/n7178/suppinfo/nature06531_S1.html

SIMMERMAN, H. K. B., & JONES, L. R. (1998). Phospholamban: Protein Structure, Mechanism of Action, and Role in Cardiac Function. *Physiological Reviews, 78*(4), 921-947.

Tilgmann, C., Pollesello, P., Ovaska, M., Kaivola, J., Pystynen, J., Tiainen, E., . . . Levijoki, J. (2013). Discovery and Structural Characterization of a Phospholamban-Binding Cyclic Peptide and Design of Novel Inhibitors of Phospholamban. *Chemical Biology & Drug Design, 81*(4), 463-473. doi: 10.1111/j.1747-0285.2012.01409.x

Volkamer, A., Eid, S., Turk, S., Jaeger, S., Rippmann, F., & Fulle, S. (2015). Pocketome of Human Kinases: Prioritizing the ATP Binding Sites of (Yet) Untapped Protein Kinases for Drug Discovery. *Journal of Chemical Information and Modeling, 55*(3), 538-549. doi: 10.1021/ci500624s

Volkamer, A., Kuhn, D., Grombacher, T., Rippmann, F., & Rarey, M. (2012). Combining Global and Local Measures for Structure-Based Druggability Predictions. *Journal of Chemical Information and Modeling, 52*(2), 360-372. doi: 10.1021/ci200454v

Volkamer, A., Kuhn, D., Rippmann, F., & Rarey, M. (2012). DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment. *Bioinformatics, 28*(15), 2074-2075. doi: 10.1093/bioinformatics/bts310

Walsh, J. D., Chu, S. D., Zhang, S. Q., & Gochin, M. (2015). Design and characterization of swapped-domain constructs of HIV-1 glycoprotein-41 as receptors for drug discovery. *Protein Engineering Design & Selection, 28*(4), 107-116. doi: 10.1093/protein/gzv006

Zhang, K., Zhang, J., Gao, Z.-G., Zhang, D., Zhu, L., Han, G. W., . . . Zhao, Q. (2014). Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature, 509*(7498), 115-118. doi: 10.1038/nature13083

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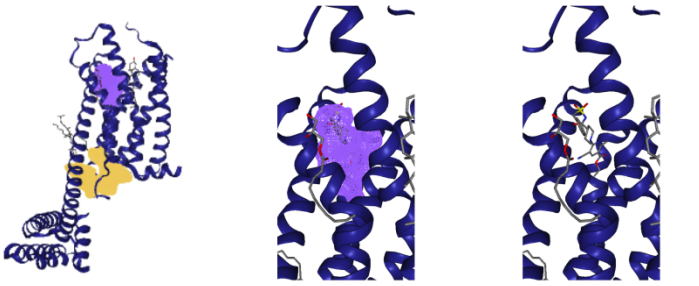
Zhang, X., Jin, L., Fang, Q., Hui, W. H., & Zhou, Z. H. (2010). 3.3 Å Cryo-EM Structure of a Nonenveloped Virus Reveals a Priming Mechanism for Cell Entry. *Cell, 141*(3), 472-482. doi: 10.1016/j.cell.2010.03.041

Zhou, G. Y., & Chu, S. D. (2013). Discovery of Small Molecule Fusion Inhibitors Targeting HIV-1 gp41. *Current Pharmaceutical Design, 19*(10), 1818-1826.



Pocket Name	Volume (Å³)	Surface (Å²)	Depth (Å)	# HB D	# HB A	# H-I	HR	Drug Score
P_0	768.23	1049.39	17.69	19	40	59	0.50	0.80
P_1	643.84	742.65	19.91	17	31	39	0.45	0.83

 # HB D: Number of Hydrogen-Bond Donor; **# HB A**: Number of Hydrogen-Bond Acceptor; **# H-I**: Number of Hydrophobic Interactions; **HR**: Hydrophobicity Ratio

Figure 1 The top two pockets of the human P2Y12 receptor (PDB code: 4NTJ) identified and analyzed by DoGSiteScorer.

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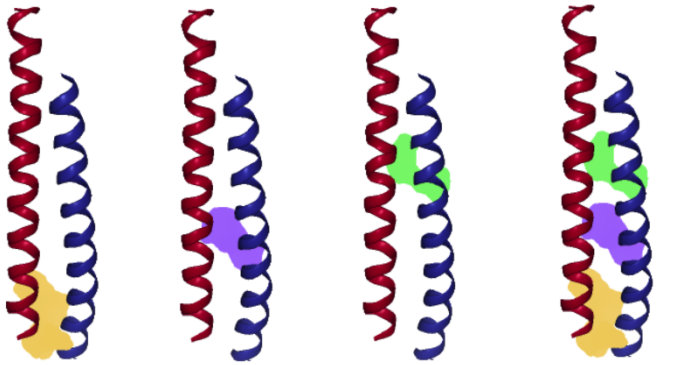

Pocket Name	Volume (Å³)	Surface (Å²)	Depth (Å)	# HB D	# HB A	# H-I	HR	Drug Score
P_0	270.78	794.05	9.27	12	22	40	0.54	0.35
P_1	141.57	396.9	8.56	6	10	21	0.57	0.27
P_2	104.19	411.32	11.83	3	12	24	0.62	0.36

 # HB D: Number of Hydrogen-Bond Donor; **# HB A**: Number of Hydrogen-Bond Acceptor; **# H-I**: Number of Hydrophobic Interactions; **HR**: Hydrophobicity Ratio

Figure 2. Druggable Pockets of GP41 (PDB code **1AIK**) identified and analyzed by DoGSiteScorer.

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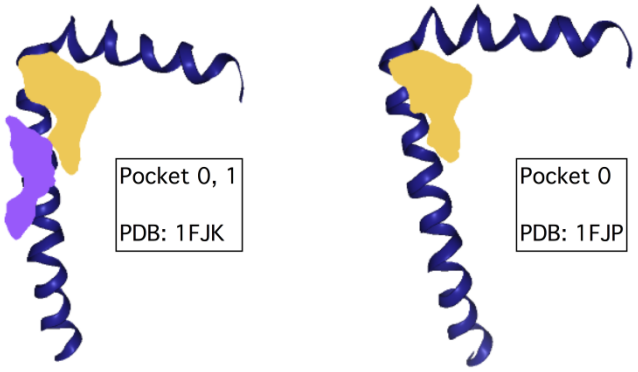

Pocket Name	Volume (Å³)	Surface (Å²)	Depth (Å)	# HB D	# HB A	# H-I	HR	Drug Score
PDB: **1FJK**, phospholamban								
P_0	326.91	762.73	15.70	4	25	48	0.62	0.67
P_1	129.86	494.64	11.95	1	10	59	0.84	0.41
PDB: **1FJP**, Phospholamban in detergent micelles								
P_0	271.3	745.48	14.46	7	27	45	0.57	0.6

 # HB D: Number of Hydrogen-Bond Donor; **# HB A**: Number of Hydrogen-Bond Acceptor;
 # H-I: Number of Hydrophobic Interactions; **HR**: Hydrophobicity Ratio

Figure 3 Druggable Pockets of Phospholamban (PLB) (PDB code **1FJK** and **1FJP**) identified and analyzed by DoGSiteScorer.

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