P2X Receptor 3D Structure Prediction Using Homology Modelling

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Abstract

P2X receptors are ATP-activated ion channels in the plasma membrane. P2X receptors have a role in a diverse range of disorders, making them a valuable therapeutic target. Hence, the present investigation employed homology modelling of the P2X receptor based on the crystal structure of 5SVJ, 6AH4, 5YVE and 5SVL. Twenty models, using both single- and multiple template-based methods, were developed, and the best model was chosen based on the validation result. We observed that a strategy based on multiple templates provided greater accuracy. Future studies involving binding site and docking analysis can make use of the produced structures.

Keyword: Homology modelling, P2X receptor

1. Introduction

P2X receptors, found in the plasma membrane, are ATP-activated ligand-gated ion channels[1]. The P2Y receptors are related to them and make up a developing family of extracellular nucleotide receptors[2]. Hence, the two subtypes of purinergic receptors, P2X and P2Y, are grouped together under the umbrella term P2 purinergic receptors, while adenosine-binding plasma membrane receptors are classified as P1 purinergic receptors[3]. P2X receptors are widely dispersed in both neuronal and glial cells across the central and peripheral nervous systems[4]. Fast transmission at central synapses, smooth muscle cell contraction, platelet aggregation, macrophage activation, and cell death are only some of the processes that are mediated by P2X receptors[5]. In addition to modulating the consequences of neuronal activity during development, neurodegeneration, inflammation, and cancer, these receptors have also been linked to integrating functional activity across neurons, glial, and vascular cells in the central nervous system[5-6]. Chronic neuropathic and inflammatory pain, depression, cystic fibrosis, dry eye, irritable bowel syndrome, interstitial cystitis, dysfunctional urinary bladder, and cancer are among the diseases being

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studied for the therapeutic potential of P2X receptor agonists and antagonists[7]. Recent insights into the role purinergic receptors in these diseases suggest the possibility of developing a drug that selectively blocks P2X receptor.

The quantity of experimentally resolved protein structures is behind the available sequencing data because of the lengthy time necessary to prepare protein for crystallisation[8]. When there is a lack of crystal structure, Homology Modeling can quickly determine the protein's three-dimensional structure from its sequence information[9]. This study used single and multiple template based homology modelling to predict the 3D structure of P2X purino receptors, which lack a crystal structure[10]. Twenty models were developed and checked with a Ramachandran plot and ERRAT values. New drugs targeting P2X receptor-based disorders could benefit from the predicted model.

2. Materials and method

2.1 Template Selection:

The P2X receptor amino acid sequence (accession no. Q00UQ1) was obtained from the Uniprot database. We looked for homology modelling starting points by performing a BLAST search on the PDB for proteins. Templates were chosen based on query coverage, E-values and sequence similarity. If the sequence identity is greater than 30%, then up to 90% of the protein conformation is usually modelled accurately. Due to rising alignment problems, models with less than 30% identity are deceitful.

2.2 Modeling and Validation of P2X Receptor:

EasyModeller 4.0[11], developed in MODELLER 9.12[12] with Python 2.7.1 as the backend, was used to model the P2X receptor's 3D structure. There were four distinct templates used for the single-template based techniques. Using the RMSD data, we analysed and verified the anticipated models.

Programs like Ramachandran plot[13] and ERRAT[14] were used later on to validate the results. Each projected model's percentage of amino acids in the preferred region is displayed graphically in a Ramachandran plot generated by the SAVES server. To gauge the success of crystallographic model construction and refinement, ERRAT, a protein structure verification algorithm, is used.

3. Results and Discussion

3.1 Template Selection

Template selection, alignment of the target with the template, model construction, and validation are all steps in the computational approach to building the model. Crystal structures 5SVJ, 6AH4, 5YVE and 5SVL were chosen as modelling templates. Table 1 displays the best blast search template results. To further investigate whether this method may enhance the model's accuracy, multiple template based homology modelling was also undertaken.

3.2 Model Generation and Validation

We used both single and multiple template approaches to construct the model. In this research, models were generated using Easy Modeller 4.0. Five models were made for each of the four different templates (5SVJ, 6AH4, 5YVE and 5SVL). As a result, this study produced twenty different models. The

root-mean-square-deviation (RMSD) of the created models compared to the templates was first determined. The generated models were analysed using ERRAT and Ramachandran plots to determine their overall quality. Table 2 summarises the outcomes of the validation. The best model within each template-based model was selected using parameters such low RMSD, high percentage of residues in favoured and allowed regions, and high ERRAT quality value. Due to its high RMSD and low ERRAT quality, 5SVL was not chosen as a template for any models. Fig. 1 depicts the final model selection criteria, which resulted in the three models depicted there, together with their respective RC plots in Fig. 2 and ERRAT plots in Fig. 3. There is just a small RMSD gap between the models we chose. The model created using many templates performed better in validation than the other two chosen models, demonstrating the superiority of the multiple-template-based strategy.

4. Conclusion

P2X receptor 3D models were generated using single template based methods. The 5SVJ and 6AH4 produced models are shown to be accurate. Increased accuracy is observed in homology modelling with multiple templates. In addition, these models have the potential benefit of being employed for binding site characterization and molecular docking in drug discovery process for P2X receptor involved disorders.

Table. 1. The identity and query coverage values of the templates.

PDB ID	Max Score	Total score	Query Coverage %	E Value	Identity %
5SVJ	339	339	76 %	3e-113	50.00 %
6AH4	338	338	75 %	4e-113	50.84 %
5YVE	338	338	75 %	4e-113	50.84 %
5SVL	333	333	76 %	5e-111	49.18 %

Table. 2. SAVES server-based validation of the generated models.

Model	Template PDB ID	Ramachandra Plot			RMSD	ERRAT- Overall quality factor	QMean Global score	ProSA web Z – score Overall model quality
		Favoured%	Allowed%	Disallowed%				
1.		91.4	7.4	0.5	0.792	36.90	–4.37	–4.38
2.		91.1	7.9	0.5	0.460	51.37	–4.67	–4.38
3.	5SVJ	**90.4**	**8.1**	**1.0**	**0.960**	**53.47**	**–5.19**	**–4.49**
4.		91.9	7.1	0.3	0.421	45.52	–4.57	–4.36
5.		92.1	6.6	0.3	0.294	41.28	–4.37	–4.7
6.		90.6	8.1	0.5	1.251	48.98	–3.69	–5.5
7.		85.7	11.8	0.5	1.386	54.7	–4.41	–5.79
8.	6AH4	**87.6**	**11.4**	**0.8**	**1.135**	**54.52**	**–5.17**	**–5.98**
9.		88.3	8.9	1.3	2.305	47.67	–4.20	–5.42
10.		87.1	10.7	0.3	1.357	48.87	–4.41	–5.61
11.		92.4	6.9	0.3	1.216	38.68	–4.94	–4.47
12.		92.4	6.6	0.5	0.632	44.80	–4.34	–4.43
13.	5YVE	89.6	9.4	0.3	0.840	46.33	–5.01	–4.45
14.		**90.9**	**7.6**	**0.8**	**0.418**	**47.68**	**–4.69**	**–4.49**
15.		90.9	8.6	0.0	0.507	46.91	–4.36	–4.94
16.		91.4	7.6	0.5	1.216	44.72	–3.65	–4.4
17.		91.9	7.1	0.3	1.799	54.45	–3.84	–4.38
18.	5SVL	91.6	7.6	0.3	1.948	51.94	–2.88	–4.47
19.		92.4	7.4	0.3	1.386	43.66	–2.94	–4.5
20.		91.9	7.4	0.0	1.720	51.66	–3.39	–4.19

(a)   (b)   (c)
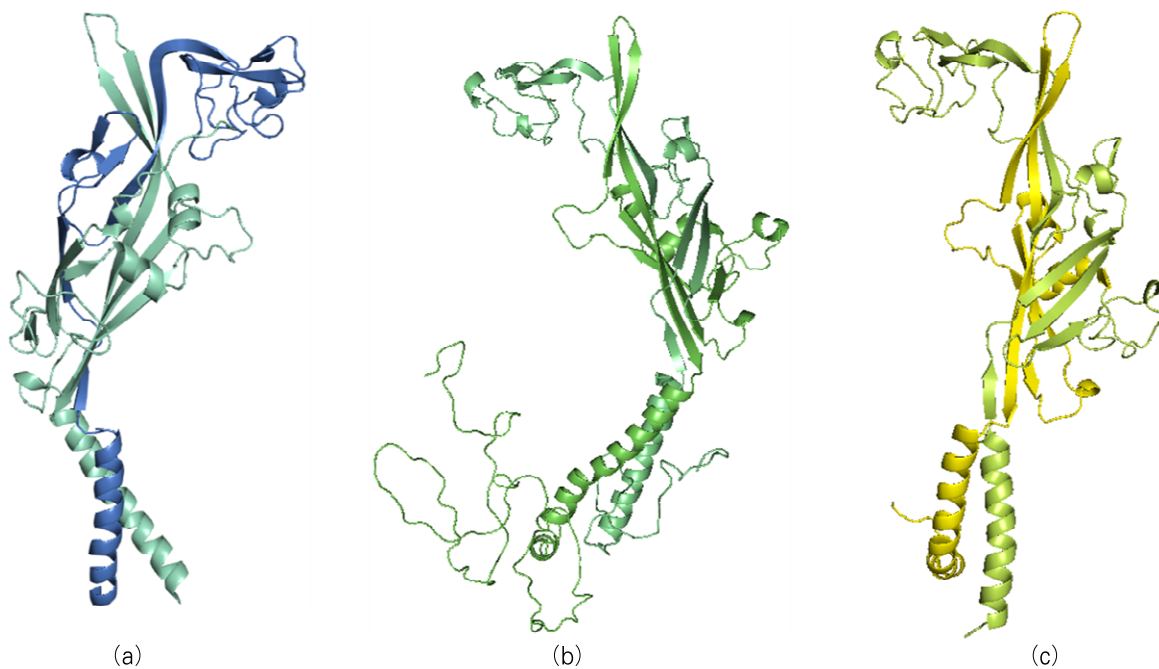
Fig. 1. Selected models for P2X purinoreceptor

Model 3   Model 8   Model 14
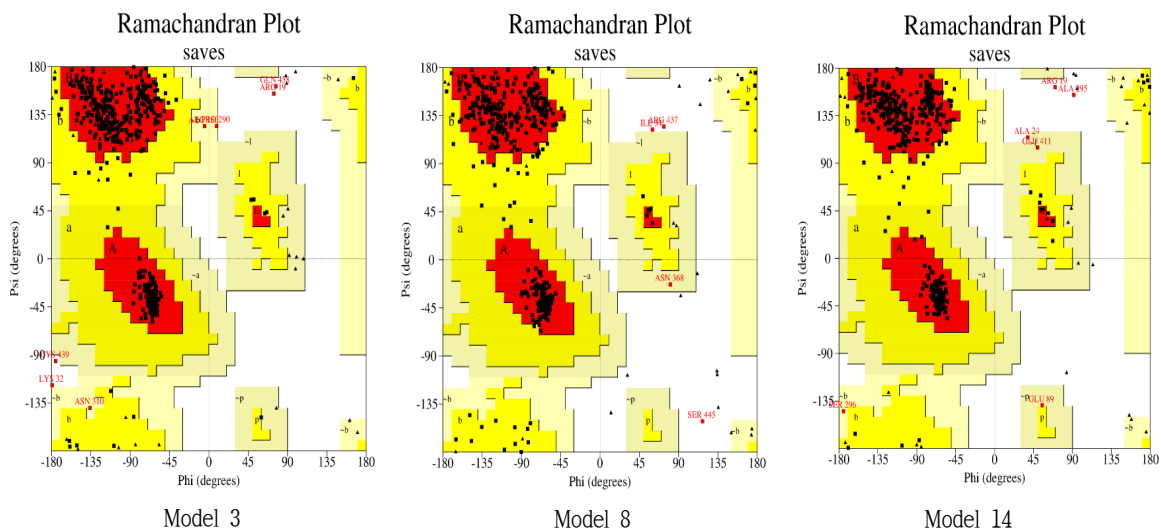
Fig. 2. Ramachandran plot for the selected models.
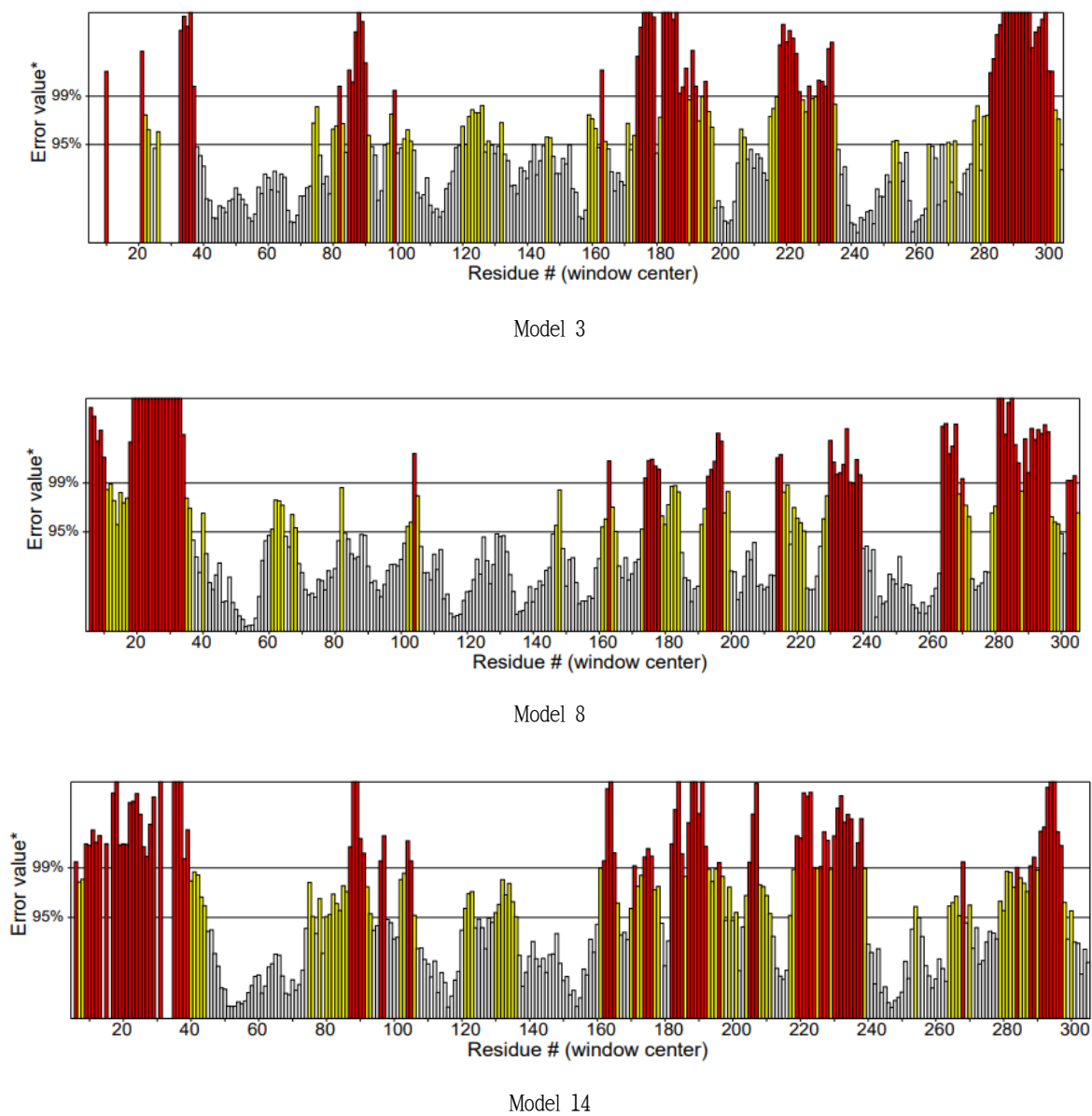
Model 3

Model 8

Model 14

Fig. 3. ERRAT plot for the selected models.

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