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# Homology modeling, agonist binding site identification, and docking in octopamine receptor of *Periplaneta americana*

Akinori Hirashima [a,*], Hongwei Huang [b]

[a] *Division of Bioresource and Bioenvironmental Sciences, Graduate School, Kyushu University, Fukuoka 812-8581, Japan*
[b] *Accelrys, 200 Wheeler Road, South Tower 2nd Floor, Burlington, MA 01803-5501, USA*

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ABSTRACT

AY333178 (from *Periplaneta americana*, 628 AAs) was selected as a target octopamine receptor (OAR) class OAR2 for this study using Discovery Studio (DS Modeling1.1/1.2, Accelrys Inc.). Blast similarity search was performed and identified that AY333178 contains N-terminal domain of GPCR. Based upon Blast and Pfam results, Rhodopsin 1U19 (protein data bank) was considered as an ideal homologue and used as a template for homology modeling due to its higher X-ray resolution at 2.2 Å. Sequence alignment between AY333178 and 1U19 was done using Align123 followed by a manual modification. The final alignment was carefully evaluated and evidenced to be matching the conserved residue data for class A GPCR fairly well. The 3D model of AY333178 was generated with MODELER, and further refined using CHARMm. Superimposition of the model was done over the template 1U19. Two fairly consistent profiles were observed demonstrating AY333178 model was reasonable and could be employed for the further docking study. Agonist docking into OAR2 model was done using LigandFit. The superimposition of two top poses of representative agonists was performed with a soft surface generated. Those models are considered to be used in designing new leads for hopefully more active compounds. Further research on the comparison of models for the agonists may elucidate the mechanisms of OAR2–ligand interactions.



## 1. Introduction

Octopamine [2-amino-1-(4-hydroxyphenyl)ethanol (OA)] is the monohydroxylic analogue of the vertebrate hormone noradrenalin. OA was first discovered in the salivary glands of octopus by Erspamer and Boretti (1951). It has been found that OA is present in a high concentration in various invertebrate tissues (Axelrod and Saavedra, 1977). This multifunctional and naturally occurring biogenic amine has been well studied and established as (1) a neurotransmitter, controlling the firefly light organ and endocrine gland activity in other insects; (2) a neurohormone, inducing mobilization of lipids and carbohydrates; (3) a neuromodulator, acting peripherally on different muscles, fat body, and sensory organs such as corpora cardiaca and the corpora allata, and (4) a centrally acting neuromodulator, influencing motor patterns, habituation, and even memory in various invertebrate species (Evans, 1985; Orchard et al., 1993). Three different OA receptor (OAR) classes OAR1, OAR2A, and OAR2B had been distinguished from non-neuronal tissues (Evans, 1981). The action of OAR2 is mediated through various messengers, which is coupled to G-proteins and is specifically linked to an adenylate cyclase (Nathanson, 1985). Thus, the physiological actions of OAR2 have been shown to be associated with elevated levels of cAMP. In the nervous system of locust *Locusta migratoria* L., a particular receptor class was characterized and established as a new class OAR3 by pharmacological investigations of the OA binding site using various agonists and antagonists (Roeder and Gewecke, 1990; Roeder, 1990, 1992, 1995).

Quantitative structure-activity relationship (QSAR) modeling is an area of research pioneered by Hansch and Fujita. The QSAR study assumes that the difference of the molecules in the structural properties experimentally measured accounts for the difference in their observed biological or chemical properties (Hansch and Leo, 1995; Hansch and Fujita, 1964). The result of QSAR usually reflects as a predictive formula and attempts to model the activity of a series of compounds using measured or computed properties of the compounds. More recently, QSAR has been extended by including the three-dimensional (3D) information. In drug discovery, it is common to have measured activity data for a set of compounds acting upon a particular protein but not to have knowledge of the 3D structure of the active site. In the absence of such 3D information, one may attempt to build a hypothetical model of the active site that can provide insight on the nature of the active site.

OAR2 has been defined as one of class A G-protein coupled receptor (GPCR). This past year has seen a steady and excit-

* Corresponding author. Tel.: +81 92 642 2856; fax: +81 92 642 2858.
*E-mail address:* ahirasim@agr.kyushu-u.ac.jp (A. Hirashima).

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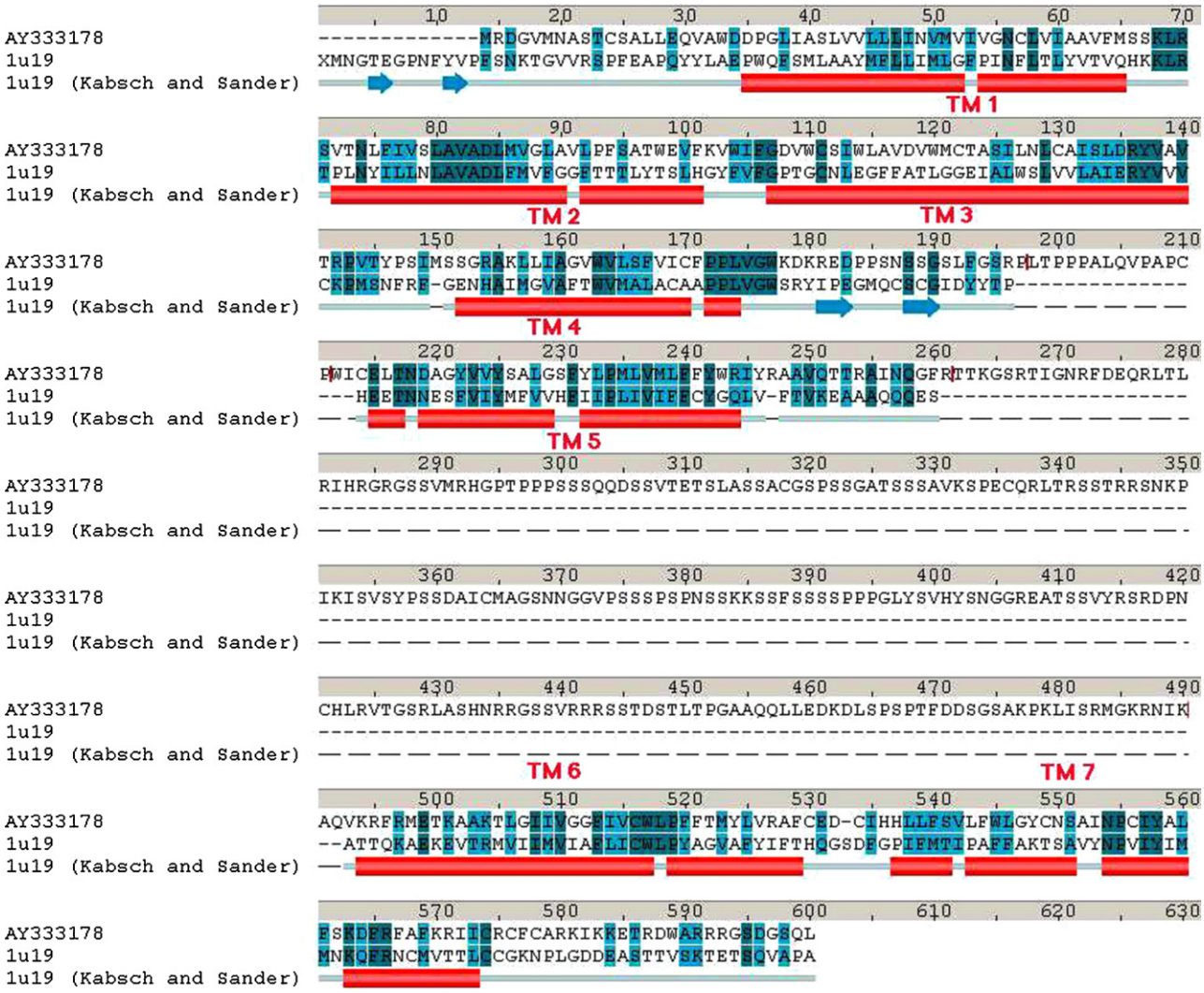


**Fig. 1.** Sequence alignment between AY333178 and 1U19 (identity, 19%; similarity, 46%).

ing growth of novel inhibitors identified through computational analysis of target structure. A combination of more structural comparison, advances in homology modeling, better docking and scoring tools, fragment-based methods, and advances in virtual screening has been fundamental in this progress. Protein structure-based small molecule design is clearly becoming a valuable and integral part of the inhibitor discovery, which has been proven to be more efficient and productive. In order to understand OAR2 protein–ligand interaction, the 3D model of OAR2 was predicted, and then its agonist binding site was identified, followed by docking study using Discovery Studio (DS Modeling1.1/1.2, Accelrys Inc.).

## 2. Materials and methods

### 2.1. Homology modeling

The homology modeling of OAR2 was performed using DS Modeling1.1. The homologue search and sequence alignment were done by two modules, sequence analysis and protein families (Align123). Sequence analysis identifies homologs for OAR2 protein sequences by searching over either NCBI (The National Center for Biotechnology Information) website, or against locally installed databases using BLAST and PSI-BLAST. Protein families calculates multiple sequence alignments using sequence and structure information, aligns sequences of OAR2 and its templates. The final 3D model was generated by MODELER, which was originally developed by Sali et al. (1995). It performed automated protein homology modeling and loop modeling for OAR2.

### 2.2. Protein simulation

OAR2 model could be further refined by CHARMm (Brooks et al., 1983) in DS Modeling1.1, which provides powerful mechanics and dynamics protocols for studying the energetics and motion of molecules, from small ligands to multi-component physiological complexes. Accelrys CHARMm forcefield was used throughout the simulation. Constraint was applied to allow only binding site

- Helix 1 : 54GNXL58V
- Helix 2 : 75FxxxLAXADL85L
- Helix 3: conserved Cys110
  124SXXXLXXIXXDR136Y
- Helix 4 : conserved Trp161 and Pro170
- Helix 5: 212FXXP(L/V/F)XXXXXX223Y
- Helix 6: 249(K/R)XX252(K/R)
  261FXXCWXP268Y
- Helix 7 : conserved Leu294
  298NSXX(N/D)PXX306Y

**Fig. 2.** Evidence of the conserved residue data in AY333178 for class A GPCR.