In silico Characterization and Docking of α-Conotoxin Mr1.7a from *Conus marmoreus* Targeting Neuronal nAChR α3β2

Rucha Wadapurkar[1], Anil Kumar Katti[2]

Department of PG studies in Bioinformatics, Walchand Centre for Biotechnology, Solapur-413006, Maharashtra, India

Abstract: *Conotoxins are a group of cysteine-rich peptide-based toxins in the venom of cone snails. The small peptides of α-Conotoxin Mr1.7a from Conus marmoreus behave pharmacologically as competitive antagonists of the nicotinic acetylcholine receptor nAChR α3β2. Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, widely spread in the central and peripheral system. nAChRs modulate the release of neurotransmitters, such as dopamine, norepinephrine, acetylcholine and γ-amino butyric acid, and they are involved in a variety of pathophysiologies, including chronic pain syndromes, epilepsy, Parkinson's and Alzheimer's. The physicochemical properties of the α-Conotoxin Mr1.7a were analyzed by using ExPASy ProtParam tool. The secondary structure prediction was done by SOPMA which showed that alpha helix dominated all the other conformations. 3D structure was predicted using modeller and validated using PROCHECK with 83.9 % found in most favoured regions [A, B, L]. VaxiJen 2.0 server predicted α-Conotoxin Mr1.7a as a non-antigenic. Binding sites were predicted using DoGSiteScorer. Docking of α-Conotoxin Mr1.7a was performed against target neuronal nAChR α3β2 using Hex 6.3 tool. The docking result with Etotal of -598.19 was provided a reasonable structural basis that can be used for future investigations on nAChR-ligand complex and α-Conotoxins serve as a best therapeutic agent.*

Keywords: α-Conotoxin Mr1.7a, *Conus marmoreus,* nAChR α3β2, DoGSiteScorer, Hex

1. Introduction

Conotoxins display a great molecular diversity, being evolved across all phylogenetic clades and feeding strategies of cone snails. This multiplicity is mirrored in the classification of at least 16 genetically distinct superfamilies where the conotoxins are categorized upon their cysteine-framework. These superfamilies are subdivided in conotoxin families depending on their impressive diversity of targets ranging from voltage-gated ion channels (sodium, potassium, and calcium) to ligand-gated ion channels (such as nicotine receptors and serotonin receptors). The implementation of this broad spectrum of pharmacologically active components has made this single genus very successful, evolving into more than 500 *Conus* species [1]. Each cone snail species produces more than 1000 conopeptides with an estimated overlap of 5% between different species [1]. Conotoxins have potential roles in the direct treatment of disease. A number of potential pharmaceuticals are being derived from conotoxins [3]. To date, only 0.1% out of potentially 500,000 venom components has been functionally and structurally investigated. Nevertheless, the consideration of *Conus* venoms as gold mines for the discovery of new therapeutics is validated by the knowledge that, out of the limited number of studied conopeptides, already six peptides have reached human clinical trials, and one which was Ziconitide, derived from Conus magus ω-conotoxin MVIIA, has been approved as analgesic in 2004 by the United States Food and Drug Administration for treating intractable pain under the brand name Prialt [3]. The toxins of *Conus* species are usually potent, selective and small (typically <5 kDa) which is an advantage for cost-effective synthesis and makes them ideal pharmacological probes, which are synthesized by cone shells from mRNA templates derived from toxin genes and expressed in the venom ducts as precursor peptides [4]. *Conus* species have evolved multiple classes of conopeptides targeting ligand-gated ion channels including nicotinic acetylcholine receptors (nAChRs), 5-hydroxytryptamine3 receptors (5-HT3Rs), and *N*-methyl-D-aspartate (NMDA) antagonists as well as α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) enhancers. Among these receptor classes, antagonists of nAChRs are the largest and most diverse.As ligand-gated ion channels, neuronal nicotinic acetylcholine receptors (nAChRs) are widely spread in the central and peripheral system [1]. nAChRs modulate the release of neurotransmitters, such as dopamine, norepinephrine, acetylcholine and γ-amino butyric acid, and they are involved in a variety of pathophysiologies, including chronic pain syndromes, epilepsy, Parkinson's and Alzheimer's [2]. α-Conotoxin Mr1.7a selectively inhibits the α3β2 neuronal nicotinic acetylcholine receptors (nAChRs).

In the present study, *in-silico* characterization and docking of α-Conotoxin Mr1.7a to neuronal nicotinic acetylcholine receptors (nAChRs) was carried out to identify α-Conotoxin as a therapeutic agent for treating epilepsy, Parkinson's and Alzheimer's diseases.

2. Materials and Methods

2.1 Sequence Retrieval

The sequence of α-Conotoxin Mr1.7a was retrieved from Uniprot database. The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data.

2.2 Analysis of physicochemical parameters

The different physicochemical properties of α-Conotoxin Mr1.7a were computed using ExPASy ProtParam tool (http://web.expasy.org/protparam/). The ProtParam includes

the following computed parameters: Molecular weight (Mol.wt), theoretical pI, instability index (II), aliphatic index (AI) and grand average of hydropathicity (GRAVY) [5].

2.3 Secondary structure prediction

The secondary structure was predicted by self-optimized prediction method with alignment (SOPMA). SOPMA was employed for calculating the secondary structural features of the selected protein sequences considered in this study. This method calculates the content of α-helix, β-sheets, turns, random coils and extended strands. SOPMA is a neural network based methods; global sequence prediction may be done by this sequence method [6].

2.4 Tertiary structure prediction

Modeller is a computer program that models three-dimensional structures of proteins and their assemblies by satisfaction of spatial restraints. Modeller is most frequently used for homology or comparative protein structure modeling: The user provides an alignment of a sequence to be modeled with known related structures and Modeller will automatically calculate a model with all non-hydrogen atoms (these structures are often homologs, but certainly don't have to be, hence the term "comparative" modeling) [8].

2.5 Assessing the quality of a tertiary structure

The Procheck is a public, Web-based resource that can be used to examine structures downloaded from the PDB in order to study the structure and function, molecular modeling, and drug-design. PROCHECK performs two functions–Precheck and Validate. The Precheck function checks the format of the files uploaded for validation, while the Validate function checks the geometry, chemistry, sequence of the structure, and computes various derived features [7].

2.6 Prediction of antigenicity

Prediction of antigenicity program VaxiJen 2.0 predicts those segments of α-Conotoxin Mr1.7a from *Conus marmoreus* that are likely to be antigenic by eliciting an antibody response. VaxiJen 2.0 antigenicity prediction tool which is the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment [8].

2.7 Binding pocket prediction

DoGSiteScorer was used to predict binding pockets in a target protein α3β2 nAChR and α-Conotoxin Mr1.7a. It is a newly developed automatic tool combining pocket prediction, characterization and druggability estimation and is now available via a web-server. DoGSiteScorer provides the functionality to detect potential binding pockets and subpockets of a protein of interest. Subsequently, it analyzes the geometric and physicochemical properties of these pockets and estimates druggability with aid of a support

vector machine. Thus, the method provides valuable information for target assessment [9].

2.8 Docking

The docking analysis of α-Conotoxin Mr1.7a and α3β2 nAChR was carried out using HEX 6.3 docking software, which is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Docking allows predicting the ligand with best scores and identifying the drug-receptor complex with lowest free energy [10].

3. Results and Discussion

3.1 Sequence Retrieval

The sequences of α-Conotoxin Mr1.7a protein of *Conus marmoreus* was retrieved from Uniprot database having accession number F5C3U4. The sequence length of α-Conotoxin Mr1.7a in *Conus marmoreus* is 69 amino acids.

3. 2 ProtParam

The physicochemical properties of α-Conotoxin Mr1.7a was predicted by using ProtParam tool. The physicochemical properties (Table 1) show that molecular weight is 7579.9 Da. The instability index (69.80) showed that the protein was unstable. The computed pI value 9.37 indicates that it was basic in nature. The very high aliphatic index inferred the better positive factor for the increase of thermostability of globular proteins. The high GRAVY index could result in less interaction with water.

Mubashshira Pathan *et. al.* (2014) in which ExPASy ProtParam tool was used to calculate physiochemical parameters of GlmU protein of *Mycobacterium tuberculosis*.

Table1: Primary structure analysis using ProtParam tool

Organism	Accession number	No. of aa	Mol.wt	pI	II	AI	Gr-avy
Conus marmoreus	F5C3U4	69	7579.9	9.37	69.80	87.68	0.316

3.3 Secondary Structure Prediction

The secondary structure prediction (Table 2) showed that alpha helix predominated the other structures and β-turn being the least conformational structure. The secondary structure indicated whether a given amino acid lies in a helix, strand or coil. Similar results were got by Mubashshira Pathan *et. al.* (2014) in which the secondary structure of GlmU antigenic protein of *Mycobacterium tuberculosis* was predicted using SOPMA.

Table 2: Percentage of amino acids sequence forming secondary structure in SOPMA prediction.

Organism name	Accession number	α- helix (Hh) (%)	B -turn (Tt) (%)	Extended strand (Ee) (%)	Rando-m coils (Cc) (%)
Conus marmoreus	F5C3U4	40.58	8.70	18.84	31.88

3.4 Modeller 9.15 and PROCHECK

The structure modeled by Modeller 9.15 (Figure 4) showed the amino acid percentage in the favourable region as 83.9% and amino acid percentage in disallowed region as 0.0% (Figure 5).

Similarly, Mubashshira Pathan *et. al.* (2014) used Modeller to generate model of GlmU protein of *Mycobacterium tuberculosis*.
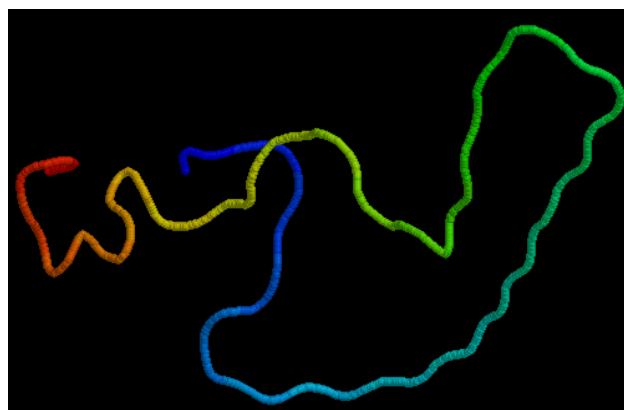

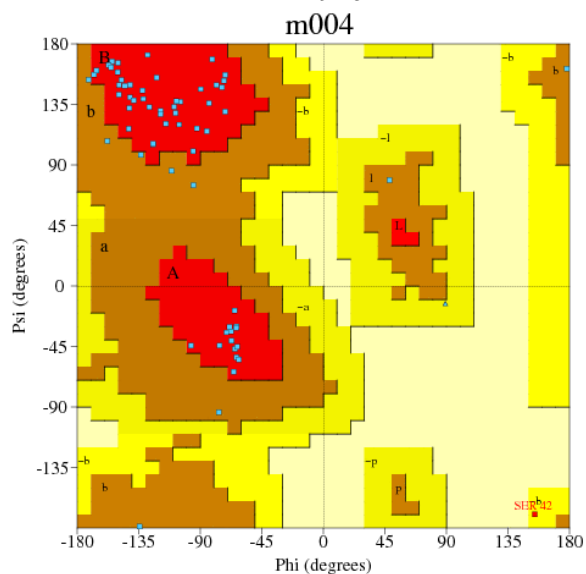**Figure 4:** 3D structure of α-Conotoxin Mr1.7a predicted by Modeller 9.15.

Figure 5: Ramchandran plot of α-Conotoxin Mr1.7a.

3.5 Antigenicity prediction

The result found that α-Conotoxin Mr1.7a was a non-antigen with overall antigen prediction score of 0.5318, predicted using VaxiJen 2.0 server, with the threshold set to 1.0 (Figure 6).

Rajashree *et. al.* (2013) reported the prediction antigenicity of *Neisseria gonorrhoeae* proteins using VaxiJen 2.0.
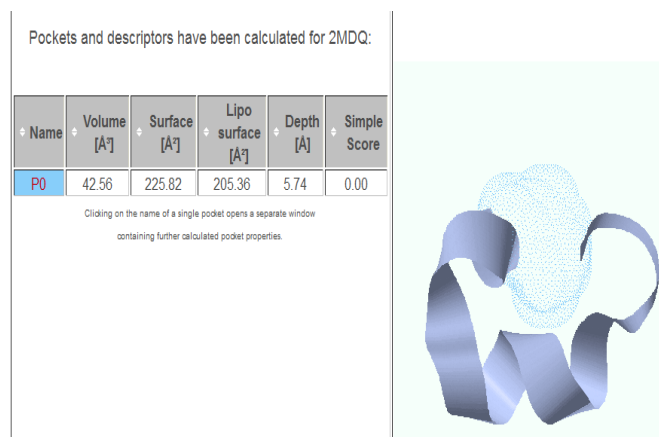

Overall Antigen Prediction = **0.5318** (Probable **NON-ANTIGEN**).

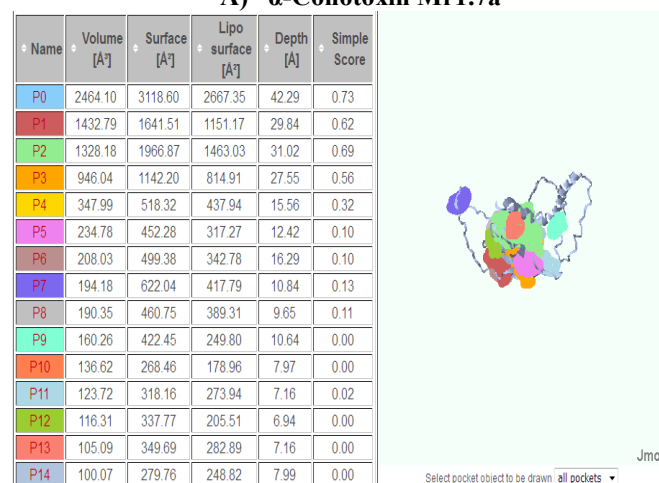**Figure 6:** Antigenicity prediction of α-Conotoxin Mr1.7a using VaxiJen 2.0.

3.6 DoGSiteScorer

The potential binding pockets of α-Conotoxin Mr1.7a were predicted using DoGSiteScorer, solely based on the protein heavy atom coordinates. One binding pocket was calculated with volume 42.56 A^3, surface 225.82 A^2, lipo surface 205.36 A^2, depth 5.74 and simple score 0.00. Also, total 15 binding pockets in α3β2 nAChR were identified (Figure 7).

Similar results were got by Andrea Volkamer *et. al.* (2012) in which binding sites of chain B of cAbl were predicted using DoGSiteScorer.

Pockets and descriptors have been calculated for 2MDQ:

Name	Volume [A^3]	Surface [A^2]	Lipo surface [A^2]	Depth [Å]	Simple Score
P0	42.56	225.82	205.36	5.74	0.00

Clicking on the name of a single pocket opens a separate window containing further calculated pocket properties.

A) α-Conotoxin Mr1.7a

Name	Volume [A^3]	Surface [A^2]	Lipo surface [A^2]	Depth [Å]	Simple Score
P0	2464.10	3118.60	2667.35	42.29	0.73
P1	1432.79	1641.51	1151.17	29.84	0.62
P2	1328.18	1966.87	1463.03	31.02	0.69
P3	946.04	1142.20	814.91	27.55	0.56
P4	347.99	518.32	437.94	15.56	0.32
P5	234.78	452.28	317.27	12.42	0.10
P6	208.03	499.38	342.78	16.29	0.10
P7	194.18	622.04	417.79	10.84	0.13
P8	190.35	460.75	389.31	9.65	0.11
P9	160.26	422.45	249.80	10.64	0.00
P10	136.62	268.46	178.96	7.97	0.00
P11	123.72	318.16	273.94	7.16	0.02
P12	116.31	337.77	205.51	6.94	0.00
P13	105.09	349.69	282.89	7.16	0.00
P14	100.07	279.76	248.82	7.99	0.00

B) α3β2 nAChR

Figure 7: Binding site prediction of (A) α-Conotoxin Mr1.7a and (B) α3β2 nAChR using DoGSiteScorer.

3.7 Hex 6.0

The Hex 6.0 docking result was found that Etotal of docking complex α-Conotoxin Mr1.7a- α3β2 nAChR was -598.19, which revealed that it had very good binding affinity and good complex formation ability (Figure 8).

Similar results were got by Vaibhav Modi *et. al.* (2013) in which docking study of anti-HIV drug BMS-488043 was performed using Hex 6.0.
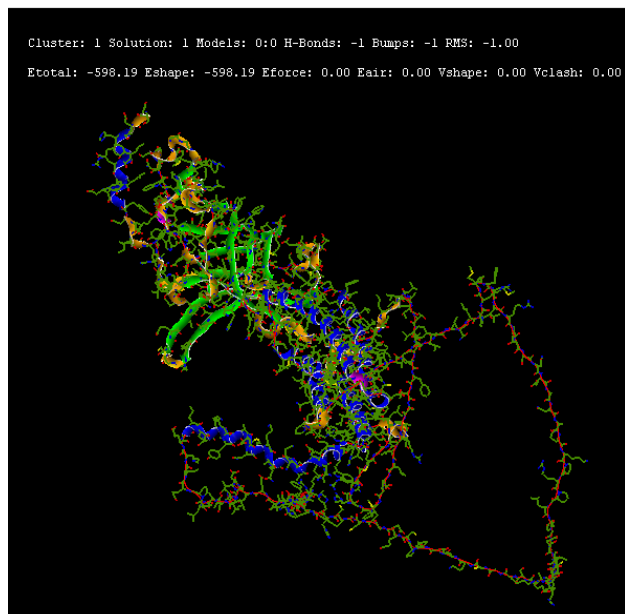
Paper ID: NOV161296   1018

Figure 8: Docking of α-Conotoxin Mr1.7a with α3β2 nAChR using Hex 6.0.

4. Conclusion

The characterization of α-Conotoxin Mr1.7a is useful for further data analysis such as different types of biomolecular interaction study, drug-likeliness and ADMET properties. The study has given an overview of the molecular pharmacology of α-conotoxin Mr1.7a selectively interacts with nicotinic acetylcholine receptor α3β2. nAChR α3β2 is implicated in the pathophysiology of a number of diseases including epilepsy, Parkinson's disease and Alzheimer's disease. This has provided a scaffold for selective peptide-engineering which can be used in drug discovery and consequently, disease treatment.

References

[1] Eline Lebbe K. M., Steve Peigneur, Isuru Wijesekara and Jan Tytgat (2014), Conotoxins Targeting Nicotinic Acetylcholine Receptors: An Overview. *Marine drugs*, (12), 2970-3004.

[2] Shuo Wang, Cong Zhao, Zhuguo Liu, Xuesong Wang, Na Liu, Weihong Du and Qiuyun Dai (2015), Structural and Functional Characterization of a Novel α-Conotoxin Mr1.7 from *Conus marmoreus* Targeting Neuronal nAChR α3β2, α9α10 and α6/α3β2β3 Subtypes. *Marine drugs*, (13), 3259-3275.

[3] Peter Anderson D. and Gyula Bokor (2012), Conotoxins: Potential Weapons from the Sea. *Bioterrorism & Biodefense*, 3(3), 2157-2526

[4] Shuo Wang, Cong Zhao, Zhuguo Liu, Xuesong Wang, Na Liu, Weihong Du and Qiuyun Dai (2009), Isolation, purification and biochemical characterization of conotoxin from *Conus figulinus Linnaeus* (1758). *Indian Journal of Biotechnology*, 8, 266-271.

[5] Swati Goli, Mayuri Burgul and Shaikh S. A (2013), *In silico* Charactrization And Comparative Analysis of PTEN in Different Species Involved in NSCLC. *Trends in Life Sciences*, 2(1), 2319–5037.

[6] Geourjon C. and Deleage G (1995), SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics*, 11 (6), 681-684.

[7] Jyotsna Choubey, Ashish Patel, Shailendra Gupta, M.K.Verma (2010), Homology Modelling Acd Binding Site Identification Of 1 Deoxy D- Xylulose 5 Phosphate Reductoisomerase Of *Plasmodium Falciparum*: New Drug Target For Plasmodium Falciparum. *International Journal of Engineering Science and Technology*, 2(8), 3468-3472.

[8] Irini Doytchinova A. and Darren Flower R (2007), VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*, 8(4), 1471-2105.

[9] Andrea Volkamer, Daniel Kuhn, Friedrich Rippmann, Matthias Rarey (2012), DoGSiteScorer: A web-server for automatic binding site prediction, analysis, and druggability assessment. *Bioinformatics Advance Access*, (2), 1-2.

[10] Vaibhav Modi, Nidhi Mathur, Amrendra Nath Pathak (2013), Molecular Docking Studies of anti-HIV drug BMS-488043 derivatives using HEX and GP120 Interaction Analysis using Pymol. *International Journal of Scientific and Research Publications*, 3(6), 1-7.

[11] Mubashshira Pathan A, Aasma Jamkhandi S. and Anil Kumar Katti S (2014), *In Silico* Characterization of GlmU Antigenic *Mycobacterium tuberculosis* protein. *IJPSR*, 5(4), 1493-1499.

[12] Rajashri Bhairamadgi N. and Anil Kumar Katti S (2013), *In-silico* Identification and Sequence Annotations of Potential Vaccine Candidate in *Neisseria Gonorrhoeae*. *International Journal of Advanced Biotechnology and Research*, 4(3), 404-414.

Author Profile

Rucha Wadapurkar pursuing M.Sc. degree in Bioinformatics in Walchand College, Department of Bioinformatics, Solapur, Maharashtra, India and received B.E. degree in Computer Science from Orchid College of Engg. and Tech., Solapur, Maharashtra, India.

Dr. Anil Kumar S. Katti received M. Sc (2003), M. Phil (2006), Ph. D (2011) in Biotechnology from Gulbarga University, Kalaburagi, Karnataka. Worked on developing lung cancer mutation database and development of histamine signaling pathway for asthma in Jubilant Biosys Pvt. Ltd. Bangalore. At present, working as Asst. Professor in Biotechnology and Bioinformatics, Walchand centre for Biotechnology, Solapur, Maharashtra, India. Area of research: Industrial Biotechnology and Bioinformatics (Drug and vaccine design).

Paper ID: NOV161296

1019