

In silico modeling of OsAAP3 transporter from *Oryza sativa* for target identification in CRISPR-Cas9 selective mutagenesis

Rodrigo Díaz¹, Gerardo Tapia², Karla Cordero³, Gabriel Donoso⁴

(1), (2), (3), (4) Instituto de Investigaciones Agropecuarias INIA, Recursos Genéticos, INIA Quilamapu, Avenida Vicente Méndez 515, Chillán, Chile.

Abstract

OsAAP3 is an aminoacid transporter (AAT) gene family member. In rice OsAAP3 transport basic aminoacid especially Lys and Arg from root to sink organs(11). The functionality decrease of OsAAP3 transporter has been related to increased efficiency in the use of nitrogen, overgrowth of buds and increased number of tillers in rice, generating better yields(12). The aim of this research is identifying the main AA residues that conform the protein and involved in the selectivity and pore interaction. All of this, in term to design a strategy to CRISPR-Cas9 mutagenesis for improve the rice yield.

Introduction and objective

Carry out molecular dynamics and Docking, to understand the functioning of one of the proteins, associated with the transport of amino acids in rice, whose loss or decrease of its functionality has been related to the increase in the efficiency in the use of nitrogen, overgrowth of buds and a greater number of tillers, generating better yields (12), in order to identify the most favorable sectors to carry out CRISPR-Cas9

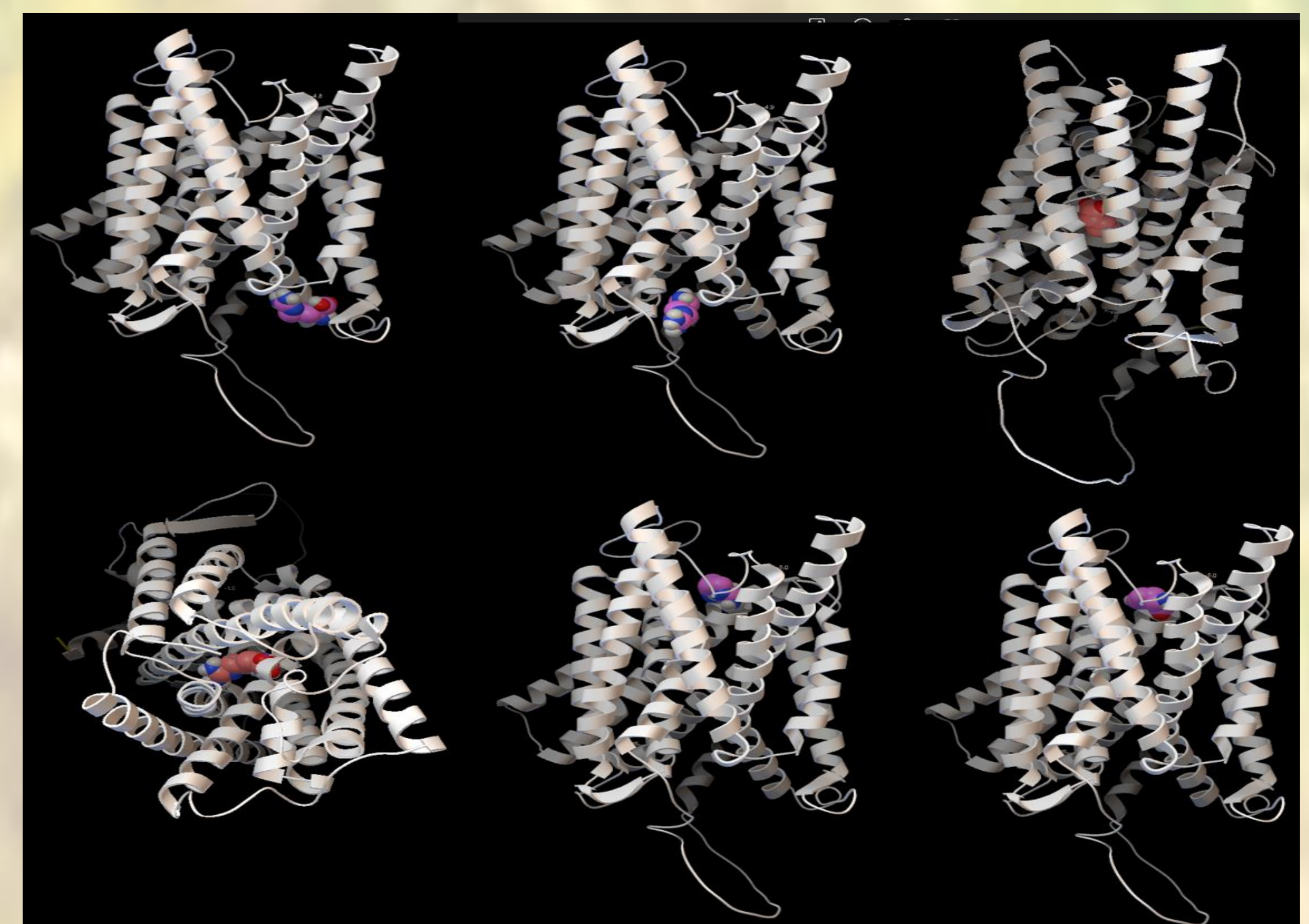
Methodology

The OsAAP3 sequence was obtained from the database <http://rice.uga.edu/> alignments were performed with Blast (1) confirming that the protein is highly conserved, PROSITE(2) and Interpro(3) protein domains were searched, the family was determined with Pfam(4) and the Obtaining the models was obtained from the modeller 10.2(5) for parts based on crystals 6c08, 7kgv and complemented with AlphaFold (13) and the homology model available in Prosite based on Q5Z9R9.

The evaluation was carried out with PROSA (8) and PROCHECK (9) and ProQ3D (10) the protein was prepared using VMD(7) and proceeded to perform a 6nm molecular dynamics with NAMD (6) version 3.0 for protein stabilization. , leaving the initial model selecting model 3 for to work for the realization of the Docking. The model corresponds to a membrane protein, which has a channel that allows the entry of amino acids.

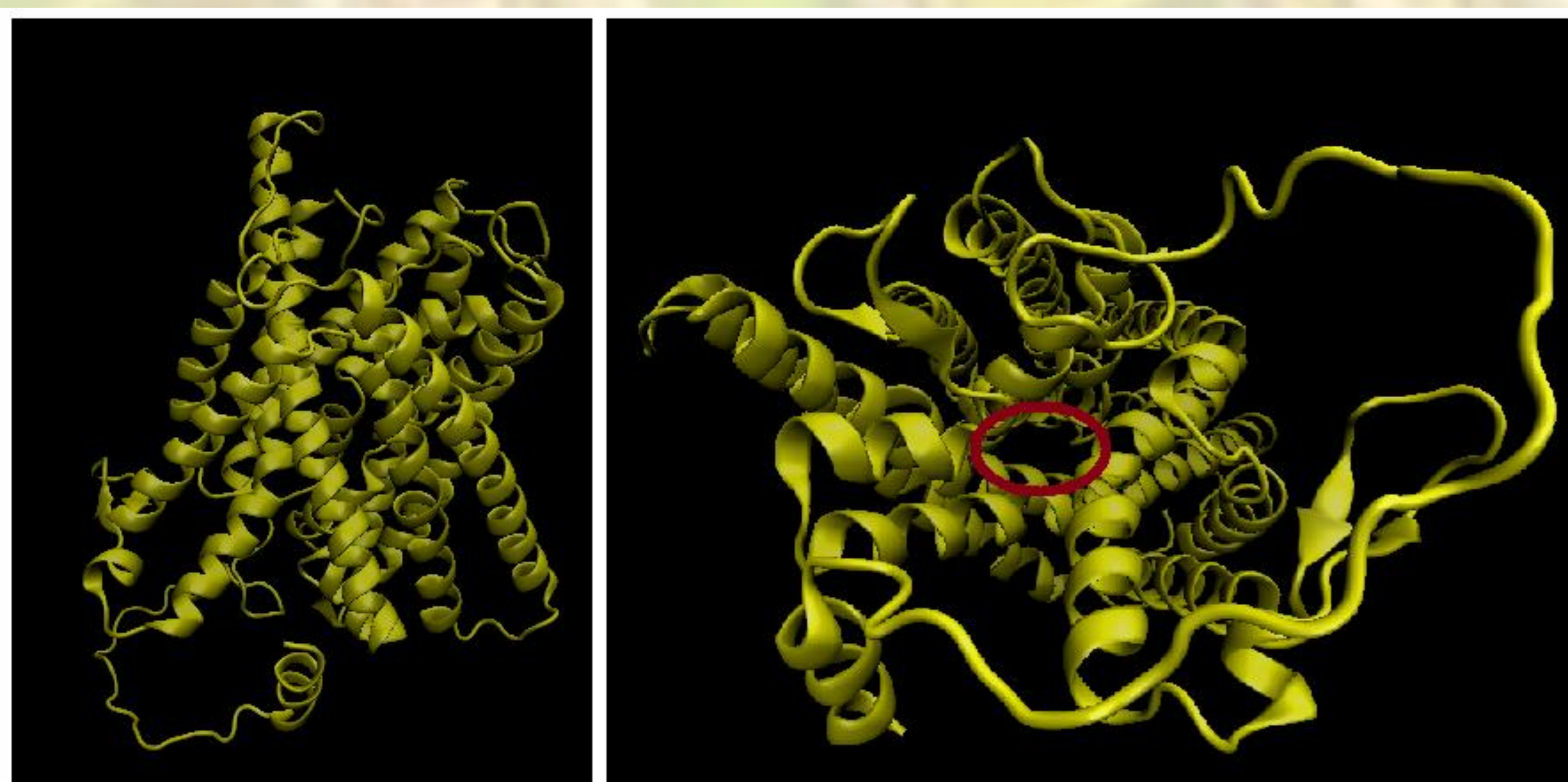


Through the docking, it was possible to detect the critical or narrowest sectors of the channel, through which the arginine could enter, in this case, several dockings were carried out, allowing the minimum energies to be obtained and seeing more or less how the arginine would move through the channel.



Discussion

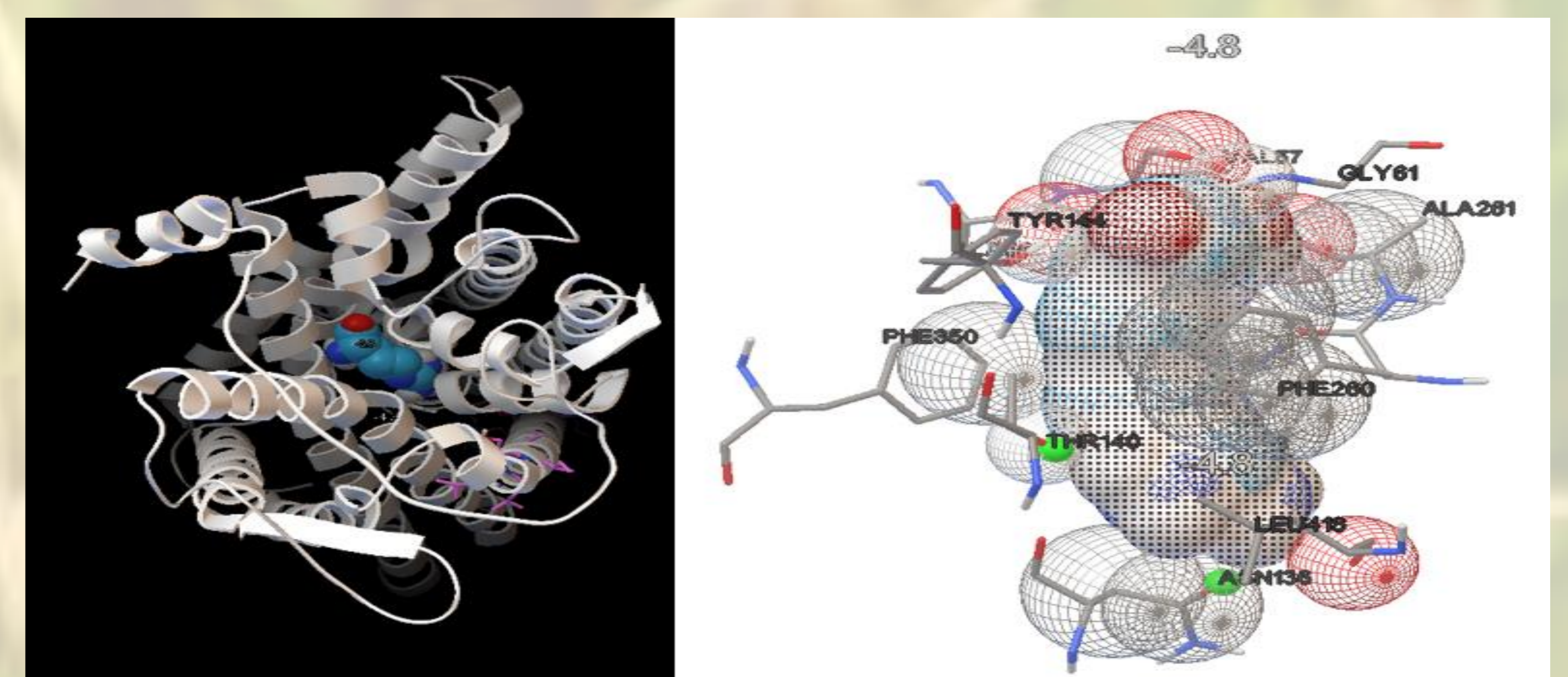
The residues proposed for crispcasp9 by (14) are exterior to the channel entry site, they can act in the conformation, for which they could be effective in a certain percentage, however the residues associated with the channel, exactly the narrowest part of this , would be the most effective in preventing the entry of amino acids, which is why they would be the most likely to be used to carry out CRISPR-Cas9, among these we can highlight two THR 140, ASN 136, as well as another six that also would serve as candidates TYR 144, VAL 57, LEU418, ALA261, GLY61, THR 140, ASN 136.



For the ligand, the arginine from the protein data bank model 2Y7I was extracted, cleaned using vmd and molecular dynamics was performed for its stabilization, leaving it ready for docking.

Results

Protein was loaded identifying the sectors studied for CRISPR-Cas9 by (14), allowing to see that these sectors were outside the transport channel.



References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, "Basic local alignment search tool.", D.J. (1990) J. Mol. Biol. 215:403-410. PubMed
- Sigrist CIA, de Castro E, Cerutti L, Cucho BA, Hulo N, Bridge A, Bougueleret L, Xenarios I. New and continuing developments at PROSITE, Nucleic Acids Res. 2012; doi: 10.1093/nar/gks1067
- Typhaine Paysan-Lafosse, Matthias Blum, Sara Chuguransky, Tiago Grego, Beatriz Lázaro Pinto, Gustavo A Salazar, Maxwell L Bileschi, Peer Bork, Alan Bridge, Lucy Colwell, Julian Gough, Daniel H Haft, Ivica Letunić, Aron Marchler-Bauer, Huaiyu Mi, Darren A Natale, Christine A Orengo, Arun P Pandurangan, Catherine Rivoire, Christian J A Sigrist, Ian Sillitoe, Narmada Thanki, Paul D Thomas, Silvio C E Tosatto, Cathy H Wu, Alex Bateman, Nucleic Acids Research (2022), gkac993, PMID: 36350672
- J. Mistry, S. Chuguransky, L. Williams, M. Qureshi, G.A. Salazar, E.L.L. Sonhammer, S.C.E. Tosatto, L. Paladin, S. Raj, L.J. Richardson, R.D. Finn, Pfam: The protein families database in 2021, A. Bateman Nucleic Acids Research (2020) doi: 10.1093/nar/gkaa913
- B. Webb, A. Sali, Comparative Protein Structure Modeling Using Modeller, Current Protocols in Bioinformatics 54, John Wiley & Sons, Inc., 5.6.1-5.6.37, 2016.
- James C. Phillips, David J. Hardy, Julio D. C. Maia, John E. Stone, Joao V. Ribeiro, Rafael C. Bernardi, Ronak Buch, Giacomo Fiorin, Jerome Henin, Wei Jiang, Ryan McGreevy, Marcelo C. R. Melo, Brian K. Radak, Robert D. Skeel, Abhishek Singharoy, Yi Wang, Benoit Roux, Aleksei Aksimentiev, Zaida Luthey-Schulten, Laxmikant V. Kale, Klaus Schulten, Christophe Chipot, and Emad Tajkhorshid, Scalable molecular dynamics on CPU and GPU architectures with NAMD. Journal of Chemical Physics, 153:044130, 2020. doi:10.1063/5.0014475
- Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38.
- Wiederstein & Sippl, ProSA-web: interactive web service for the recognition of errors in three-dimensional, (2007)
- Laskowski R A, MacArthur M W, Moss D S, Thornton J M, PROCHECK - a program to check the stereochemical quality of protein structures. (1993). J. App. Cryst., 26, 283-291.
- Karolis Uziela, David Menéndez Hurtado, Nanjiang Shu, Björn Wallner, Arne Elofsson, ProQ3D: improved model quality assessments using deep learning, Bioinformatics, Volume 33, Issue 10, 15 May 2017, Pages 1578–1580, <https://doi.org/10.1093/bioinformatics/btw819>
- Kai Lu, Bowen Wu, Jie Wang, Wei Zhu, Haipeng Nie, Junjie Qian, Blocking amino acid transporter OsAAP3 improves grain yield by promoting outgrowth buds and increasing tiller number in rice, Weiting Huang and Zhongming Fang, Plant Biotechnology Journal (2018) 16, pp. 1710–1722
- Function, transport, and regulation of amino acids: What is missing in rice? Nan Guo, Shunan Zhang, Mingji Gu, Guohua Xu, The Crop Journal 9 (2021) 530–542. doi:10.1016/j.cropro.2020.101811. PMID: 33671705; PMCID: PMC7927093.
- Jumper, J., Evans, R., Pritzel, A. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021). <https://doi.org/10.1038/s41586-021-03819-2>
- Wei Q, Yan Z, Xiong Y, Fang Z. Altered Expression of OsAAP3 Influences Rice Lesion Mimic and Leaf Senescence by Regulating Arginine Transport and Nitric Oxide Pathway. Int J Mol Sci. 2021 Feb 22;22(4):2181. doi: