Boron Uptake in Salt Cedars via Aquaporins

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Background

Salt Cedar (*Tamarix*) is a dicot plant highly tolerant to the chemical boron. For most plants boron is an essential yet toxic metalloid. Plants have a hard time excluding it. The goal of the project is to identify a potential protein sequence (order of amino acids forming a protein) for an aquaporin that allows the transport of boron, moving through a pore. In addition to selecting the sequences, a 3D model of the protein has been constructed to see how boron is entering the cells through the channels of these proteins. A dynamic model is being made to examine the structure in a cell membrane.

Molecule	Water	Urea	Boric Acid
Radius (Å)	1.4	1.8	2.3

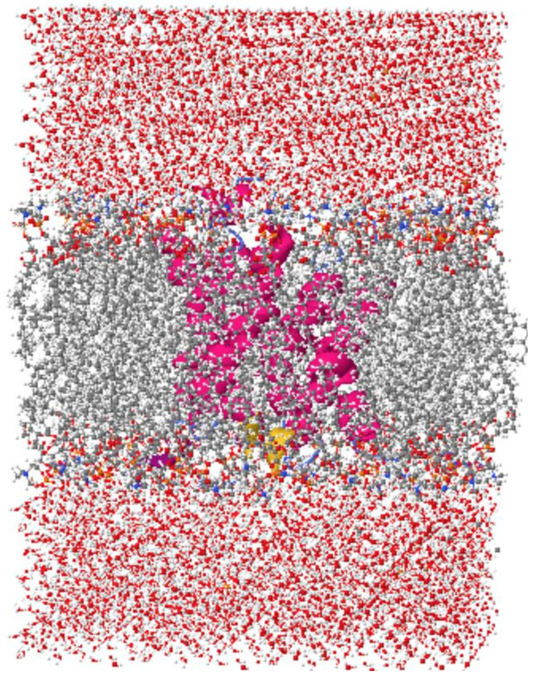
Methods

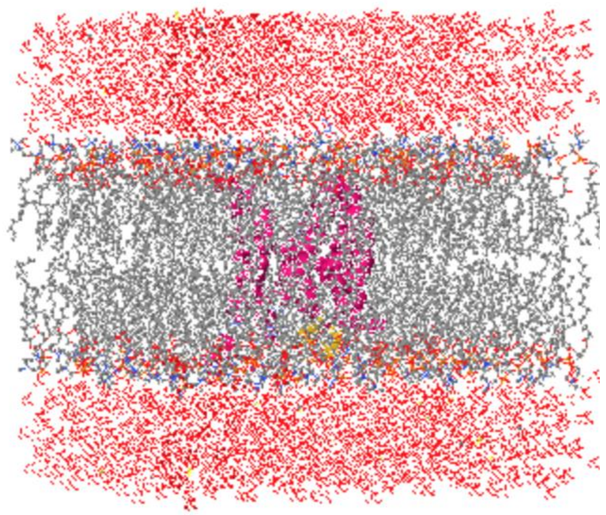
In addition to selecting the sequences, a 3D model of the protein has been constructed to see how boron is entering the cells through the channels of these proteins. A dynamic model is being made to examine the structure in a cell membrane. We have assembled 3D models of these channel proteins using computer software programs that build models based on the sequences. The models are based on *Pichia pastoris* Aquaporin 1 structure (P.p.AQY1). The models of *Arabidopsis thaliana* (A.t.) and *Cucumis sativus* (C.s.) were then analyzed in a second program to determine the size of the protein's channel. The known structure of *Homo sapiens* Aquaporin 1 (H.s.AQP1) was used to determine the validity of programs' results. The diameter of the channel/pore is a critical value being calculated. Molecular dynamic simulations are being run on these models as well. (See flowchart for molecular dynamics steps.)

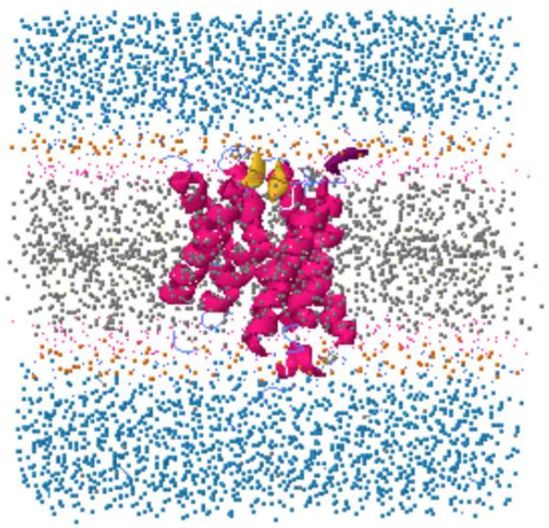
Results

The radius of the channel is a critical value; it determines whether or not boron fits through the protein channel. The results of the sequence *C.s.N.I.P.5;1* (closest sequenced relative found) did not show a large enough radius to allow the movement of boron through the protein in a static model. The nearly identical *A.t.N.I.P.5;1* has been shown to move boron, yet also does not show a large enough pore radius either. This raises the question of how dynamic the pore channel must be to allow boron to pass through. In the dynamic model the pore should have a larger size at times. The pore size will determine if boron will fit through the channel. We expect this channel to have a lower presence in the roots of this plant, thus limiting boron uptake.

Pore Size	HsAQP1	PpAQY1	AtNIP5;1	CsNIP5;1
Radius (Å)	1.24	0.5	0.91	0.91







Figures- Left: Salt cedars in the laboratory. **Middle Top:** Fine grain molecular dynamics system of *C.s.N.I.P.5;1* monomer using CHARMM-GUI Membrane Builder Module. **Middle Bottom:** Course grain molecular dynamics system of *C.s.N.I.P.5;1* monomer using CHARMM-GUI PACE CG Bilayer Builder Module. **Top Right:** All atom course grain model using CHARMM-GUI PACE CG to All-atom Converter Module.

Bibliography

- Akcay, Ufuk C. and Erkan Ibrahim E. Silicon Induced Antioxidative Responses and Expression of BOR2 and Two PIP Family Aquaporin Genes in Barley Grown Under Boron Toxicity. Plant Mol Biol Rep (2016) 34:318–326 DOI 10.1007/s11105-015-0923-5.
- B. R. Brooks, C. L. Brooks III, A. D. Mackerell, L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York, and M. Karplus: CHARMM: The Biomolecular simulation Program, J. Comp. Chem. 30, 1545-1615 (2009).
- Chovancova, E., Pavelka, A., Benes, P., Strnad, O., Brezovsky, J., Kozlikova, B., Gora, A., Sustr, V., Klvana, M., Medek, P., Biedermannova, L., Sochor, J. Damborsky, J. (2012) CAVER 3.0: A Tool for the Analysis of Transport Pathways in Dynamic Protein Structures. PLoS Comput Biol 8:e1002708. doi:10.1371/journal.pcbi.1002708
- E.L. Wu, X. Cheng, S. Jo, H. Rui, K.C. Song, E.M. Dávila-Contreras, Y. Qi, J. Lee, V. Monje-Galvan, R.M. Venable, J.B. Klauda, and W. Im (2014) CHARMM-GUI Membrane Builder Toward Realistic Biological Membrane Simulations. J. Comput. Chem. 35:1997-2004
- Gerhard Fischer, Urszula Kosinska-Eriksson, Camilo Aponte-Santamaría, Madelene Palmgren, Cecilia Geijer, Kristina Hedfalk, Stefan Hohmann, Bert L. de Groot, Richard Neutze, Karin Lindkvist-Petersson. Crystal Structure of a Yeast Aquaporin at 1.15 Å Reveals a Novel Gating Mechanism. June 16, 2009. https://doi.org/10.1371/journal.pbio.1000130
- Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38.
- J. Lee, D.S. Patel, J. Ståhle, S-J. Park, N.R. Kern, S. Kim, J. Lee, X. Cheng, M.A. Valvano, O. Holst, Y. Knirel, Y. Qi, S. Jo, J.B. Klauda, G. Widmalm, and W. Im (2019) CHARMM-GUI Membrane Builder for Complex Biological Membrane Simulations with Glycolipids and Lipoglycans. J. Chem. Theory Comput. in press
- James C. Phillips, Rosemary Braun, Wei Wang, James Gumbart, Emad Tajkhorshid, Elizabeth Villa, Christophe Chipot, Robert D. Skeel, Laxmikant Kale, and Klaus Schulten. Scalable molecular dynamics with NAMD. Journal of Computational Chemistry, 26:1781-1802, 2005.abstract, journal (NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.)
- Junpei Takano, Motoko Wada, Uwe Ludewig, Gabriel Schaaf, Nicolaus von Wirén, Toru Fujiwara. The Arabidopsis Major Intrinsic Protein NIP5;1 Is Essential for Efficient Boron Uptake and Plant Development under Boron Limitation. The Plant Cell Jun 2006, 18 (6) 1498-1509; DOI: 10.1105/tpc.106.041640
- Kelley LA *et al.* The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols* 10, 845-858 (2015).
- Lu Zhange, Jiapei Yan, Olena K. Vatamaniuk, and Xiangge Du. CsNIP2;1 is a Plasma Membrane Transporter from Cucumis sativusthat Facilitates Urea Uptake When Expressed in Saccharomyces cerevisiae and Arabidopsis thaliana. Plant Cell Physiol (2016) 57 (3): 616-629. DOI: https://doi.org/10/1093/pcp/pcw018
- NCBI. Cucumis sativus strain:9930, Cucumis sativus Refseq. 2009 October. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA182750. NCBI:BioProject: PRJNA182750.
- Qingpo Liu, Huasen Wang, Zhonghua Zhang, Jiasheng Wu, Ying Feng, and Zhujun Zhu. (2009), Divergence in function and expression of the NOD26-like intrinsic proteins in plants. BMC Genomics 10:313. DOI: 10.1186/1471-2164-10-313
- Reid, Rob. Understanding the boron transport network in plants. Plant Soil (2014) 385:1–13. DOI 10.1007/s11104-014-2149-y
- S. Jo, J.B. Lim, J.B. Klauda, and W. Im (2009) CHARMM-GUI Membrane Builder for Mixed Bilayers and Its Application to Yeast Membranes. Biophys. J. 97:50-58
- S. Jo, T. Kim, and W. Im (2007) Automated Builder and Database of Protein/Membrane Complexes for Molecular Dynamics Simulations. PLoS ONE 2(9):e880
- S. Jo, T. Kim, V.G. Iyer, and W. Im (2008) CHARMM-GUI: A Web-based Graphical User Interface for CHARMM. J. Comput. Chem. 29:1859-1865
- S. Jo, X. Cheng, S.M. Islam, L. Huang, H. Rui, A. Zhu, H.S. Lee, Y. Qi, W. Han, K. Vanommeslaeghe, A.D. MacKerell, Jr., B. Roux, and W. Im (2014)CHARMM-GUI PDB Manipulator for Advanced Modeling and Simulations of Proteins Containing Non-standard Residues. Adv. Protein Chem. Struct. Biol. 96:235-265
- Stephen F. Altschul, John C. Wootton, E. Michael Gertz, Richa Agarwala, Aleksandr Morgulis, Alejandro A. Schäffer, and Yi-Kuo Yu (2005) "Protein database searches using compositionally adjusted substitution matrices", FEBS J. 272:5101-5109.
- Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.
- USDA, NRCS. 2017. The PLANTS Database (http://plants.usda.gov, 31 March 2017). National Plant Data Team, Greensboro, NC 27401-4901 USA.
- Wallace IS, Roberts DM. Homology Modeling of Representative Subfamilies of Arabidopsis Major Intrinsic Proteins. Classification Based on the Aromatic/Arginine Selectivity Filter. Plant Physiology. 2004;135(2):1059-1068. doi:10.1104/pp.103.033415.
- Y. Qi, X. Cheng, W. Han, S. Jo, K. Schulten, and W. Im (2014) CHARMM-GUI PACE CG Builder for Solution, Micelle, and Bilayer Coarse-Grained Simulations. J. Chem. Inf. Model. 54:1003–1009

Molecular Dynamics Design Steps using CHARMM-GUI → Select PDB model to use and define unit cell → Select system model to construct → Design bilayer with lipids and insert protein → Select energy minimization constraints and forcefields. → Equilibrate model and run simulation