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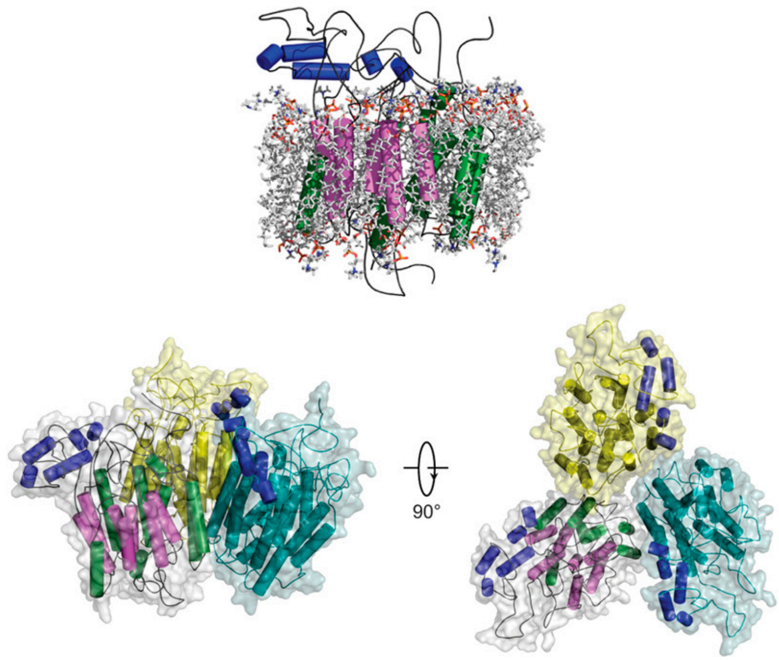
IN BRIEF

# Integrative Study Reveals Sodium Dependence of a Barley Borate Transporter

Membrane transporters are fundamental to the survival of plants; they mediate water and nutrient uptake from the soil, govern the distribution of solutes throughout the plant, and remove toxins from the cytosol. Given their gatekeeping functions, membrane transporters are attractive targets in efforts to increase nutrient and reduce toxin concentrations in the edible parts of crop plants. By affecting the uptake of potentially toxic elements such as heavy metals and metalloids into plant cells, they can also directly affect crop yields. However, manipulating the uptake or export of specific nutrients through membrane transporters relies on a detailed understanding of the transporters' molecular functions (Schroeder et al., 2013), and since these transporters are embedded in lipid membranes, this is no mean feat. Indeed, only three plant transporter proteins have been characterized at the structural level to date.

Now, **Nagarajan et al. (2016)** have developed a multidisciplinary approach to characterize the borate efflux transporter Bot1 from barley (*Hordeum vulgare*). Boron toxicity affects crop yields in a number of areas in the world, and Bot1 is known to play a key role in tolerance to high levels of boron in the soil (Sutton et al., 2007). However, its three-dimensional structure and the molecular basis of permeation are unknown.

The authors used a combination of biochemical and molecular biology techniques to show that Bot1 is localized to the plasma membrane and exists in mono-, di-, and trimeric forms, the latter being the most abundant. They then used in silico structural homology modeling combined with molecular dynamics simulations to develop an atomistic model of Bot1 and supported this model with atomic force microscopy measurements. They predicted that Bot1 folds into a three-dimensional structure consisting of 13 transmembrane and five cytosolic $\alpha$-helices (see figure) and that seven of the transmembrane $\alpha$-helices shape a single pore. The authors next evaluated the transport functions of Bot1 using voltage- and patch-clamp techniques in *Xenopus laevis* oocytes expressing *Bot1* and in liposomes harboring cell-free-synthesized Bot1, respectively. Whereas Bot1 was able to transport various anions, it was by far the most permeable to borate. Molecular dynamics simulations in a lipid bilayer environment revealed two potential sodium binding sites on the cytoplasmic side of Bot1. Intriguingly, the anion transport function of Bot1 was shown to depend on the presence of sodium ions in the medium and Bot1 conductance was abolished when key residues in one of the sodium binding sites were mutated. The authors hypothesized that specific residues in the sodium binding site and a defined sodium hydration state create an energy barrier that permits efficient anion transport by Bot1, possibly through a quantum tunneling process.




Predicted structure of Bot1. Atomistic model of Bot1 embedded in a dioleoylphosphatidylcholine bilayer that is 28 to 29 Å thick (top) and its trimeric assembly viewed along the membrane plane (bottom left) or from the cell interior (bottom right). Transmembrane $\alpha$-helices are shaded in pink and green, and intracellular $\alpha$-helices are in blue. The image was created using PyMOL. (*Adapted from Nagarajan et al. [2016]*, *Figures 3C and 3E*.)

Based on these findings, the authors concluded that Bot1 is a sodium-dependent, channel-like polyvalent anion-permeable transporter, with a high selectivity for borate anions. In addition to elucidating the transport mechanism of Bot1, this study presents an approach for deciphering the molecular functions of other membrane transporters and paves the way for developing crops that have improved nutritional properties and maintain high yields in a variety of suboptimal soil conditions.

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## REFERENCES

**Nagarajan, Y., et al.** (2016). A barley efflux transporter operates in a $Na^{+}$-dependent manner, as revealed by a multidisciplinary platform. Plant Cell **28:** 202–218.

**Schroeder, J.I., Delhaize, E., Frommer, W.B., Guerinot, M.L., Harrison, M.J., Herrera-Estrella, L., Horie, T., Kochian, L.V., Munns, R., Nishizawa, N.K., Tsay, Y.-F., and Sanders, D.** (2013). Using membrane transporters to improve crops for sustainable food production. Nature **497:** 60–66.

**Sutton, T., Baumann, U., Hayes, J., Collins, N.C., Shi, B.J., Schnurbusch, T., Hay, A., Mayo, G., Pallotta, M., Tester, M., and Langridge, P.** (2007). Boron-toxicity tolerance in barley arising from efflux transporter amplification. Science **318:** 1446–1449.