In silico prediction of the structure of the VAMP72a SNARE protein of *Lotus japonicus*, critical for forming mycorrhizal interactions

Konrad Ślusarz

Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

Introduction

LjVAMP72a and LjVAMP72b are *Lotus japonicus* proteins of the R-SNARE family. As discovered by Sogawa A. et al (2019), their presence in *L. japonicus* is a critical factor in the formation of root nodule symbiosis (RNS), an interaction between plants (primarily legumes) and nitrogen-fixing bacteria, as well as symbiosis with arbuscular mycorrhizal fungi (AMF), during which AMF penetrate cortical cells of a plant's root to achieve two-way transfer of substances – photosynthesis products from the plant and mineral nutrients from the fungus. The absence of the two LjVAMP72 proteins in mutants was shown to have a considerable negative impact on the frequency of formation of these interactions, as well as other negative effects on the plant's development and health.

LjVAMP72a, examined here, is a VAMP72 – a green-plant-specific VAMP7 (Vesicle-Associated Membrane Protein 7), a type of R-SNARE (aRginine-contributing Soluble N-ethylmaleimide-sensitive factor-Attachment Protein (SNAP) REceptor) which is found on transport vesicles, in the case of LjVAMP72a transporting signalling substances to the membrane of the *Lotus* cell to be carried to the organisms involved in RNS and to AMF.

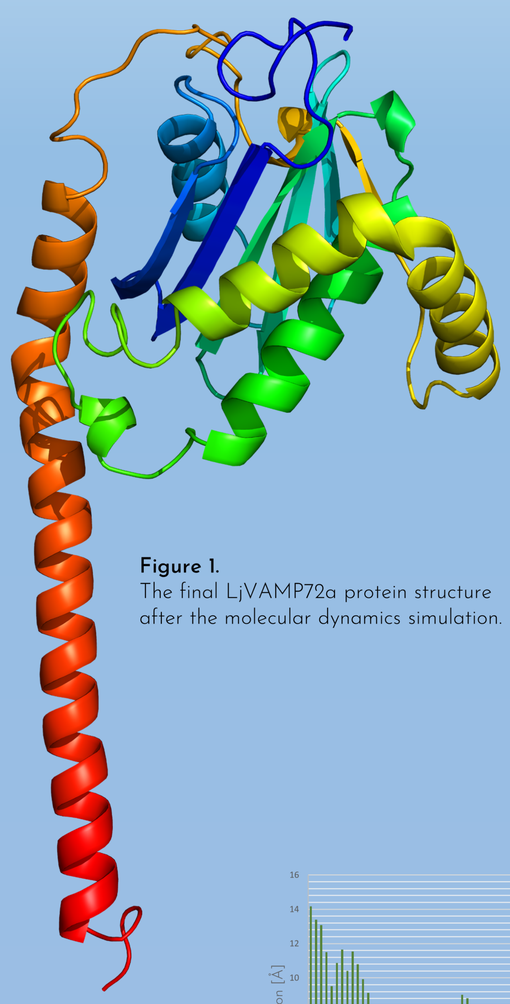


Figure 1.
The final LjVAMP72a protein structure after the molecular dynamics simulation.

Methods

The structure of LjVAMP72a was initially approximated by a SWISS-MODEL server using AlphaFold v2, based on the soybean (*Glycine max*) protein I1MFZ3, whose sequence similarity with LjVAMP72a is 58% and its coverage 96%*. Because of only partial coverage, the initial model was optimised, first by manually appending the missing amino acids at the N-terminus and C-terminus of the model and then adding all missing hydrogen atoms in the LEaP module of AMBER.

A membrane was built around the bitopic α-helix of LjVAMP72a using the PACKMOL-Memgen workflow from AmberTools, along with the *lipid21* and *ff14SB* force fields. The system was then hydrated with TIP3P water molecules, and the total charge was neutralised with Cl^- counterions.

The entire system was energy-minimised, after which 50 nanoseconds of an unrestrained molecular dynamics (MD) simulation in the NPT ensemble with periodic boundary conditions were performed. During MD, long-range electrostatic interactions were computed with the particle-mesh Ewald (PME) summation.

After obtaining the final structure (Figure 1), additional trajectory analyses were performed using CPPTRAJ.

*The connection is worth noting that *Glycine max* is also a plant known for developing determinate root nodules, like *Lotus japonicus*.

Figure 2. Root-mean-square deviation

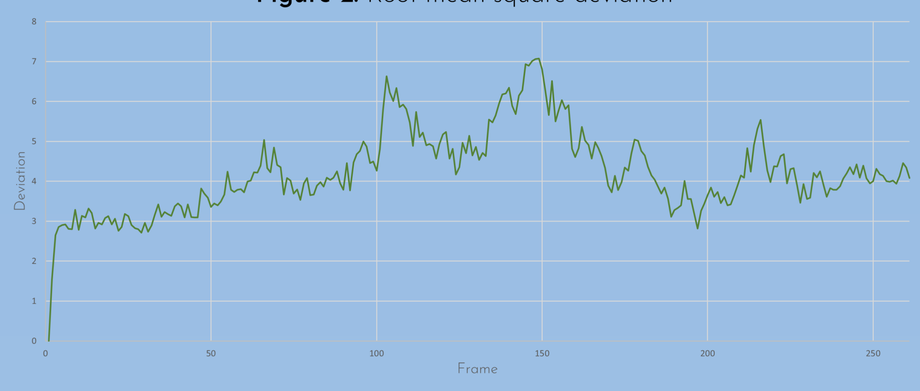
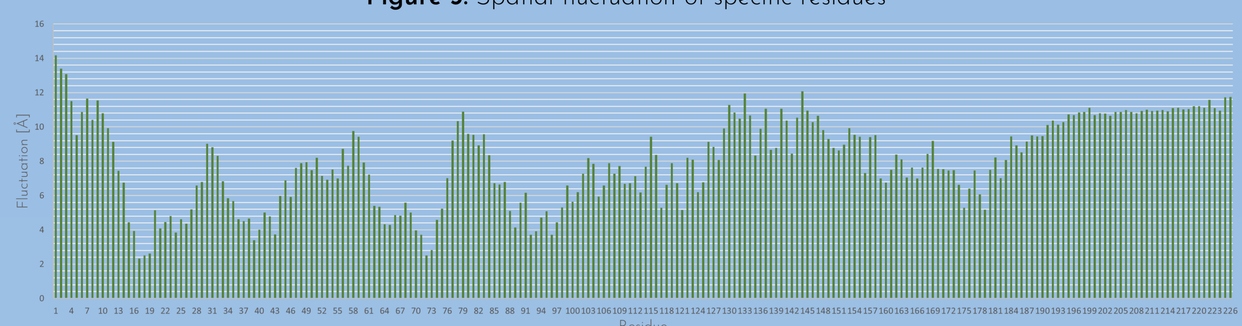


Figure 3. Spatial fluctuation of specific residues



Results

Over the course of the simulation, the structure of LjVAMP72a shifted considerably, but ended up stabilising, as is visible in the plot of root-mean-square deviation over time (Figure 2).

It can also be noted with the aid of the RMS fluctuation plot (Figure 3) how the C-terminal part of the protein, which constitutes the α-helix anchor, shifts consistently together, as opposed to the rest of the protein, which undergoes noticeable and profound changes in structure.

Finally, a noteworthy phenomenon can be observed when examining the secondary structure over time plot (Figure 4), and specifically the N-terminal fragment (Figure 5), where several amino acids near the N-terminus clearly appear to be folding into a 3_{10} helix fragment.

Figure 4.
Secondary structure over frame of simulation, whole protein (res. 1-226).

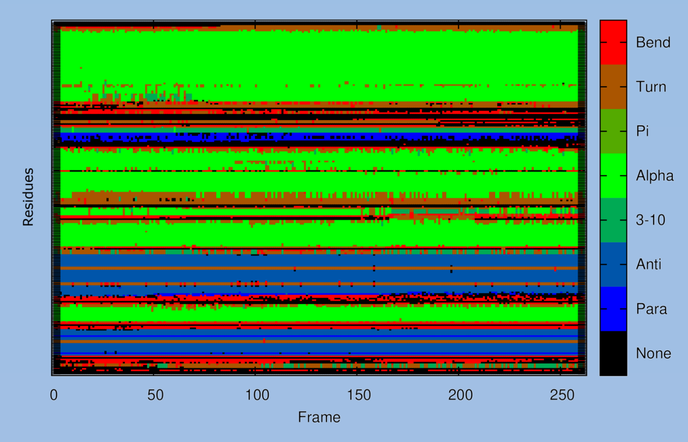
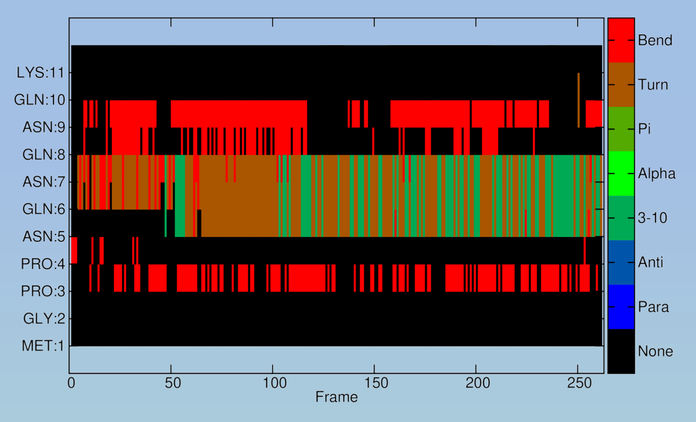
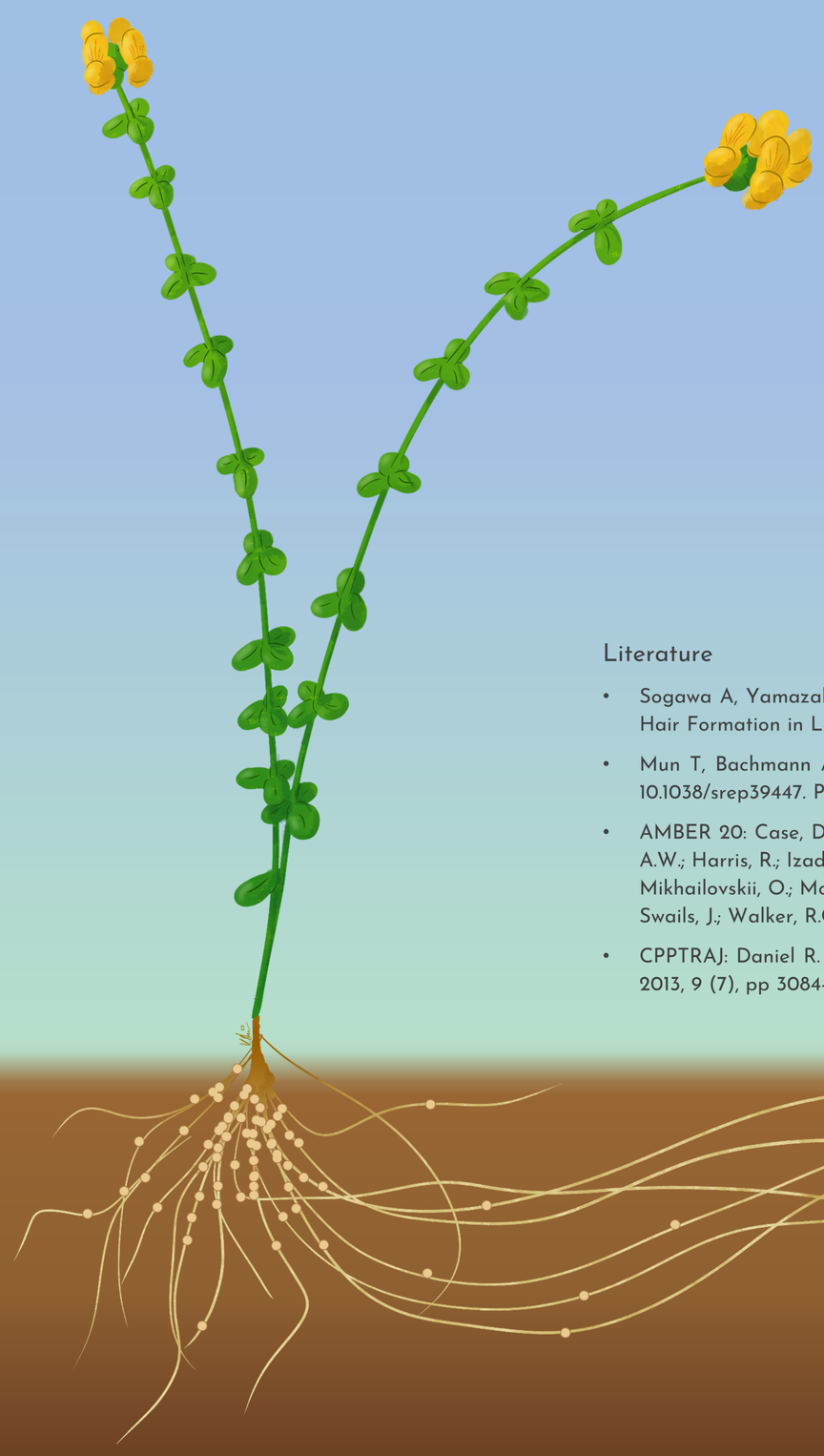


Figure 5.
Secondary structure over frame of simulation, residues 1-11.



Conclusions

- The probable structure of LjVAMP72a was determined.
- The extramembranous portion of LjVAMP72a underwent the largest changes in structure over the course of MD – consistent with its specificity's importance to the formation of the zero ion layer in its SNARE complex.
- The N-terminal end of LjVAMP72a, which did not have an analogous fragment in the reference protein, clearly shows signs of helix formation.



Literature

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