Report for Blue Waters Illinois Project Allocation

Project Information

Project Title. Unraveling the Molecular Magic of Witchweed.

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Executive Summary

Parasitic weeds of genera Striga, commonly called witchweed, are considered the most damaging agricultural agent in the developing world. An essential step in parasitic seed germination is sensing of a family of plant hormones called strigolactones, which are released by the host plants. Despite the economic importance of strigolactones, little fundamental information is known about this plant hormone. The recently obtained crystal structures of strigolactone receptor form provided a unique opportunity to explore the functional mechanism of strigolactone signaling in plants. Using computational time on Blue Waters, we investigated the *apo* and *holo* strigolactone receptor in the *Arabidopsis* plant through molecular dynamics simulations. We are able to identify multiple intermediate states as well as significant differences in the activation mechanism of *apo* and *holo* receptors. Our findings pave the path for the molecular design for chemical control of witchweed infestations.

Description of research activities and results

Key Challenges. Witchweed is a root parasite that is considered a serious agricultural pest affecting crops such as sorghum, maize (corn), rice, millet and cowpea among other crops. Witchweed seeds remain in the soil for decades, until favorable germination conditions are provided by a host plant. These seeds sense host-exuded stimulant molecules, a plant hormone called strigolactones (SLs), and start growing rapidly by attaching themselves to the root of the host plant and competing for nutrients with the crops. The parasite continues to grow beneath the soil, hence undetected, and the emergence of flowering shoots occurs months later by when the crop has been completely destroyed leading to huge economic damage. Currently, there is a need for a control technique to combat the further outbreak of this menacing parasite. In *Arabidopsis thaliana*, strigolactones are identified by the receptor protein D14. Upon identification/binding to the receptor, the SLs undergo hydrolysis and several experimental groups have reported potential activation mechanisms for D14 protein. The key challenge involves assessing the effect of the ligand produced after the hydrolysis reaction on the activation process of the receptor protein. The available crystal structures[1,2] provide only some of the snapshots of the stable protein conformations that will enable the community to understand the conformational change associated with inactive (open) to the

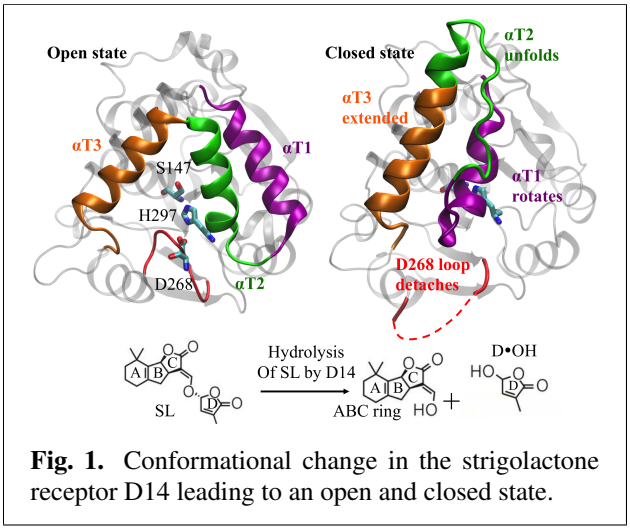

Fig. 1. Conformational change in the strigolactone receptor D14 leading to an open and closed state.

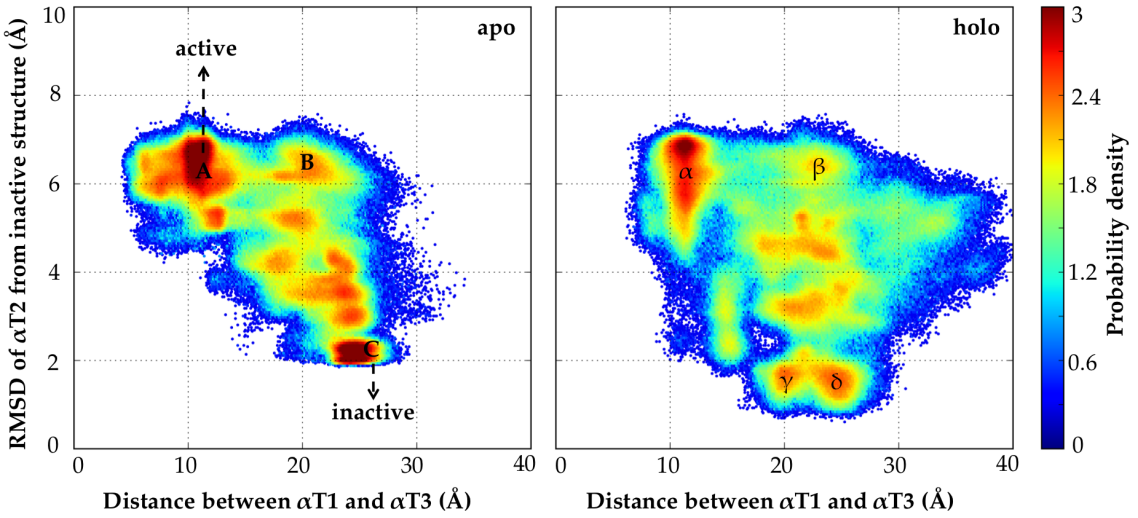

Fig. 2. Probability density maps of *apo* and *holo* D14 Strigolactone receptor based on simulation data.

active (closed) state (Fig. 1). To enable the discovery of the various intermediates involved in the activation process, specifically, we now performed extensive molecular dynamics (MD) simulations of the *apo* and covalently linked intermediate molecule (CLIM) *holo At*D14 starting from the available crystal structures, for approx. 190 and 120 μs respectively. The conformational changes associated with unfolding of helix αT2, and closing in of αT1 towards αT3 are expected to occur over long-timescales. Hence, large amount of unbiased simulation times have to be accessed in order to characterize the activation mechanism.

Why it matters. The lack of molecular level insights in the SL perception and SL induced activation of the D14 protein; and any computational studies thereof, has prevented the development of agrochemicals for controlling the spread and growth of parasite. These fundamental questions can be answered using atomistic Molecular Dynamics (MD) simulations. The present MD simulation study on Blue Waters is necessary for developing a chemical control mechanism for the witchweed parasite and to prevent billion dollar worth of annual agricultural losses.

Why Blue Waters. Understanding the slow conformational transitions in proteins requires hundreds of microsecond-long simulations. Blue Waters provides the state of the art computer architecture needed to perform such studies. We employ large scale adaptive sampling protocols, which can be efficiently performed on the Blue Waters Supercomputer GPU and CPU framework. The current work could not be possible without Blue Waters.

Accomplishments. The simulation results mapped into a two-dimensional conformational landscape of αT1-αT3 distance verus versus the root mean squared deviation of the αT2 with respect to the inactive structure (PBD ID: 4IH4[1]) shows that closed (active) state is more accessible in *holo* and hence, multiple activation pathways may be possible. We find that the *apo* protein can also exhibit active-like conformations (region A in Fig. 2) for which no previous crystal structure is known. However, the active state is extremely difficult to escape in *apo*, whereas in *holo*, there are multiple minima (regions indicated by γ and δ in Fig. 2). The simulation data was used to build Markov state models (MSMs) of conformational dynamics by clustering the structurally similar conformations into states and obtaining the interconversion rates between

these states from the simulated trajectories.

Next Generation Work. Our future work will focus on the activation mechanism of the strigolactone receptor in the presence of multiple hydrolysis reaction products which have been reported in literature. Since the conformational change study will require microseconds long simulations, we will use Blue Waters to run thousands of MD simulations of the receptor protein. Our goal is to characterize the behavior of the protein at the molecular level and pin-point the difference in activation in the host and the parasitic plant species' protein.

List of publications, datasets associated with this work

1. Mittal S*, Chen J*, Selvam B and Shukla D. Leveraging computational simulations to understand the activation mechanism of strigolactone receptor in *Arabidopsis thaliana*. Manuscript in preparation, 2018

2. Chen Q, Mittal S, Shamsi Z and Shukla D. Molecular dynamics simulations reveal the conformational heterogeneity of a strigolactone receptor in parasitic plants. Manuscript in preparation, 2018.

References

1. Zhao, L.-H. *et al.* Crystal structures of two phytohormone signal-transducing α/β hydrolases: karrikin-signaling KAI2 and strigolactone-signaling DWARF14. *Cell research* **23**, 436–439 (2013).

2. Yao, R. *et al.* DWARF14 is a non-canonical hormone receptor for strigolactone. *Nature* (2016).