# UNRAVELING THE MOLECULAR MAGIC OF WITCHWEED

**Allocation:** Illinois/300 Knh
**PI:** Diwakar Shukla[1]

[1]University of Illinois at Urbana-Champaign

## EXECUTIVE SUMMARY

Parasitic weeds of the genus Striga, commonly called witchweed, are considered the most damaging agricultural agents in the developing world. An essential step in parasitic seed germination is the 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 receptors 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 receptors in the *Arabidopsis thaliana* plant through molecular dynamics simulations. We were able to identify multiple intermediate states as well as significant differences in the activation mechanisms of *apo* and *holo* receptors. Our findings pave the way toward molecular design for the chemical control of witchweed infestations.

## RESEARCH CHALLENGE

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 undetected. The emergence of flowering shoots occurs months later, by which time the crop has been completely destroyed, leading to huge economic damage. Currently, there is a need for a control technique to combat the 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.

Several experimental groups have reported potential activation mechanisms for the 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 from the inactive (open) to the active (closed) state (Fig. 1). To enable the discovery of the various intermediates involved in the activation process, we performed extensive molecular dynamics (MD) simulations of the *apo* and covalently linked intermediate molecule (CLIM) *holo* A*t*D14 starting from the available crystal structures, for

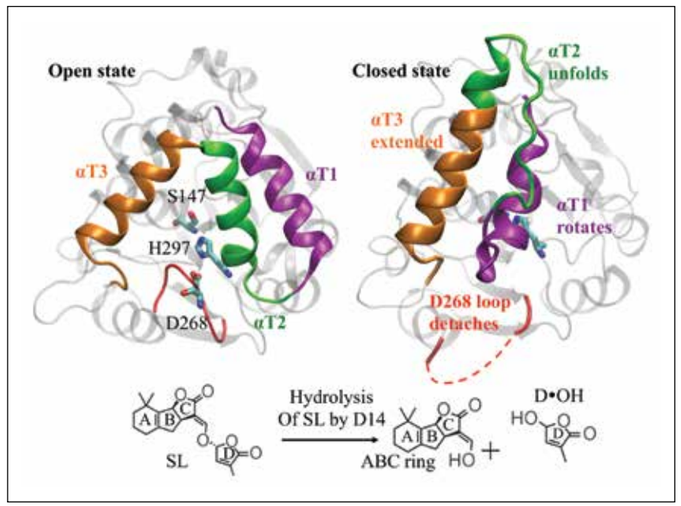


Figure 1: Conformational change in the strigolactone receptor D14 leading to an open and closed state.





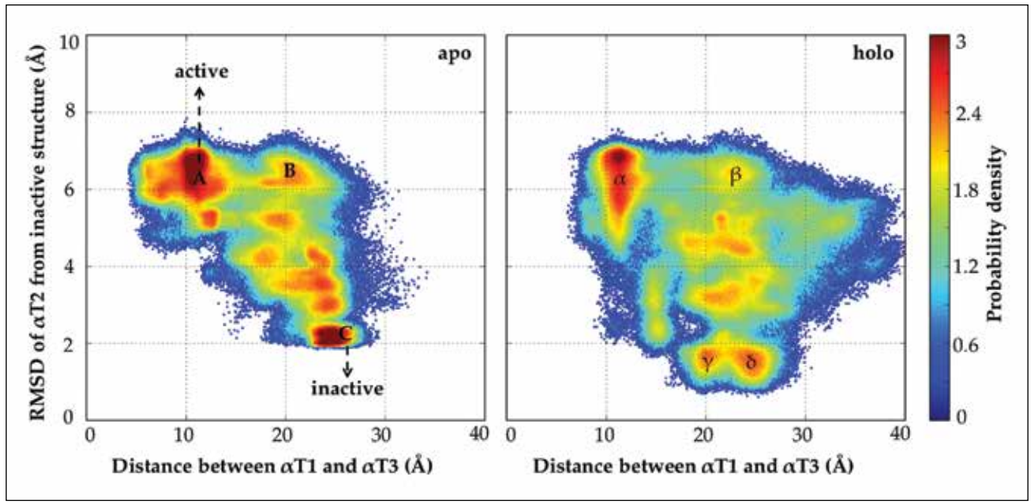


Figure 2: Probability density maps of *apo* and *holo* D14 strigolactone receptor based on simulation data.

approximately 190 and 120 microseconds, respectively. The conformational changes associated with the unfolding of helix αT2 and the closing in of αT1 toward αT3 are expected to occur over long timescales. Therefore, large amounts of unbiased simulation times have to be accessed in order to characterize the activation mechanism.

## METHODS & CODES

The MD systems were neutralized by adding a 0.15M concentration of sodium and chloride ions. All MD systems were subjected to minimization and equilibration for 2 nanoseconds on CPUs. The production runs were performed on one Blue Waters GPU node at 300 K and 1 bar. To find more informative starting points for the second round of simulations that can speed up the exploration of the conformational landscape, we used the adaptive sampling approach in the study.

## RESULTS & IMPACT

The lack of molecular-level insights regarding SLs and SL-induced activation of the D14 protein, as well as the lack of any computational studies, has prevented the development of agrochemicals for controlling the spread and growth of this parasite. However, these fundamental questions can be answered using atomistic 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 a billion dollars' worth of annual agricultural losses.

The simulation results mapped into a two-dimensional conformational landscape of the αT1–αT3 distance versus the root mean squared deviation of the αT2 helix with respect to the inactive structure (PBD ID: 4IH41) show that the closed (active) state is more accessible in *holo*; hence, multiple activation pathways may be possible. We found 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). We used the simulation data to build Markov state models of conformational dynamics by clustering the structurally similar conformations into states and obtaining the interconversion rates among these states from the simulated trajectories.

Our future work will focus on the activation mechanism of the SL receptor in the presence of multiple hydrolysis reaction products that have been reported in the 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 pinpoint the difference in activation in the host and the parasitic plant species' protein.

## 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 Blue Waters' GPU and CPU framework. The current work would not be possible without Blue Waters.