COMPUTATIONAL INVESTIGATION OF DROUGHT-RESISTANCE IN PLANTS

Allocation: Illinois/300 Knh
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EXECUTIVE SUMMARY

With the Earth's rising population and changes in the global climate, the biggest challenge facing humanity will be meeting future food and energy needs. While photosynthetic plants are our principal source of food and biofuels, we still know little about how plants adapt to environmental stresses. Plant kinases and phosphatases have been identified as the key signaling enzymes involved in regulation of photosynthetic efficiency and response to external stresses, but the molecular understanding of these stress and energy signaling enzymes remains elusive. Using Blue Waters, we investigated the conformational dynamics of plant receptor-like kinases involved in Brassinosteroid signaling and conducted a methodological study using evolutionary information to guide protein simulations. We have identified several key plant kinases whose activity can potentially be regulated via conformational engineering of αC-helix. These kinases are involved in key processes related to plant growth and development, such as regulating nutrient transport and drought-tolerance.

INTRODUCTION

Increase in demand for our primary foodstuffs is outstripping increase in yields, an expanding gap that indicates large potential food shortages by mid 21st century [1, 2]. This comes at a time when yield improvements are slowing or stagnating as the approaches of the Green Revolution reach their biological limits [3]. With the threat of global climate change and frequent occurrence of extreme weather events such as droughts, the task of producing sufficient food and biofuels is expected to become even more challenging in the future [4]. Plants respond to changing environmental conditions by translating extracellular signals (typically in the form of small molecules such as hormones) into appropriate intracellular responses. Cell-surface receptor-like kinases (RLKs) play a major role in extracellular sensing and transmitting the information into the cytoplasm for downstream signaling [5, 6]. In plants, the receptor-like kinases play key roles in regulating growth and development, protection against pathogens, and reproductive success in generating seeds and fruits and hindering premature abscission. Therefore, a quantitative molecular-level understanding of these plant-signaling processes is fundamental to future food and energy security. We focus on one of the best-characterized Leucine-rich repeat (LRR) RLK in plants, Brassinosteroid-insensitive--1 (BRI1), the receptor for plant steroid hormones called Brassinosteroids, which are crucial for plant growth.

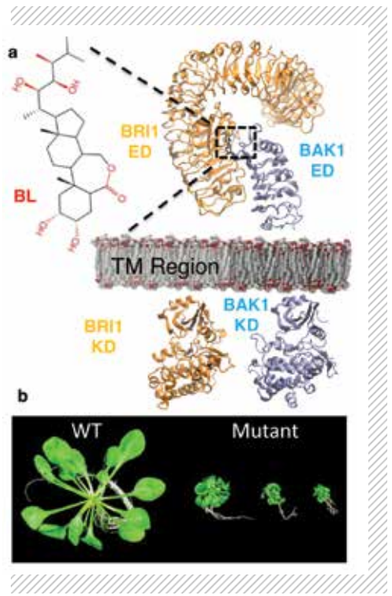

FIGURE 1: a) Crystal structures of the extracellular domains (ED) bound to brassinolide (BL) and the cytoplasmic kinase domains (KD) of AtBRI1 and AtBAK1 RLKs. b) Arabidopsis thaliana wild-type (WT) and dwarf plants from the BL deficient mutants.

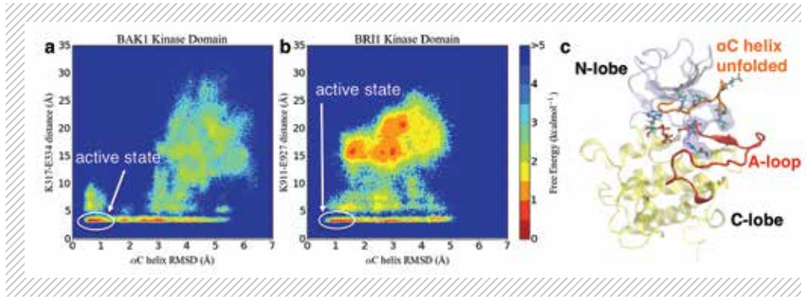

FIGURE 2: Free energy landscape of fully phosphorylated, ATP-bound kinase domain as a function of αC helix RMSD from the crystal structures and K317 side-chain amine nitrogen to E334 side-chain carboxyl carbon distance for a) BAK1 b) BRI1 kinase. c) Structure of BRI1 kinase domain with a αC-helix in unfolded state.

METHODS & RESULTS

The key aims of this project were to computationally investigate Brassinosteroid signaling from hormone reception to regulation of the BR activity using Blue Waters. Specifically, we have now performed extensive molecular simulations of the BAK1 and BRI1 receptor kinase domains starting from the available crystal structures. The simulation data was used to build Markov state models (MSMs) of kinase dynamics by clustering the structurally similar conformations into states and obtaining the interconversion rates between these states from the simulated trajectories. Such detailed thermodynamic (stability of a particular conformation of protein or a protein-protein complex) and kinetic (rate of interconversion among different conformations) information about the underlying conformational free energy landscape has provided new insights into the regulation of BAK1 and BRI1 kinase function.

The crystal structures of the fully phosphorylated BAK1 and BRI1 kinase domain show a folded αC-helix conformation. However, simulations reveal completely unfolded conformations of the αC-helix. The folded conformation of the αC-helix is a critical feature of the active state as the catalytically important K317-E334 h-bond is disrupted in the unfolded state. Fig. 2 shows regions with an unfolded αC-helix (high αC root mean square deviation (RMSD) from the folded structure) and a broken K-E h-bond. A simulation snapshot of the BRI1 kinase domain with an unfolded αC-helix is also shown (Fig. 2c). These results indicate the presence of catalytically incompetent conformations in the ensemble of kinase domain in its fully phosphorylated state. We have also performed CD experiments on BAK1 and BRI1 kinases to validate the computational prediction of the unstable αC-helix region. Finally, we have performed *in silico* mutagenesis to design BAK1 and BRI1 kinase domains with stable a αC-helix, thereby enhancing the catalytic activity of kinases. Our investigations reveal that Somatic Embryogenesis Receptor Kinase (SERK) family kinases show large differences in their αC-helix unfolding propensity, which could provide new avenues for conformational control of kinase activity of individual SERK-family kinases.

WHY BLUE WATERS

Slow conformational transitions are difficult to observe by running simulations on commodity hardware. Powerful resources like Blue Waters are required to study such complex biological processes in full atomistic detail and over long timescales. Blue Waters provides thousands of GPUs that are used for parallel molecular dynamics simulations to perform Markov state model-based adaptive sampling of conformational energy landscape of proteins. Blue Waters increases the overall compute performance by **several orders of magnitude** (in terms of the real time required for simulation).

PUBLICATIONS AND DATA SETS

Shukla, D. *et al.*, Conformational Heterogeneity of the Calmodulin Binding Interface. *Nature Communications* 7, 2016, doi:10.1038/NCOMMS10910

Shukla, S., Z. Shamsi, A. Moffett, B. Selvam and D. Shukla. Application of Hidden Markov Models in Biomolecular Simulations. *Methods in Molecular Biology*, In press, 2016.

Moffett, Z. Shamsi, and D. Shukla. Guiding functional dynamics and dimerization of proteins using evolutionary couplings. Submitted, *Nature Communications*, 2016.