Predicting effective targets for novel antimicrobials: probing an essential signalling network in Burkholderia pseudomallei as a test case

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Project description:
Bacterial survival depends upon their ability to respond to potential threats such as environmental stresses, attack by the host's immune system and antibiotics. Threats are detected using sensors (kinases), which trigger responses and ensure survival. Some bacteria have hundreds of sensors and while most of these sensors work independently (Figure 1A), the more important decisions affecting processes such as survival, virulence and antibiotic resistance, can rarely be made based on a single signal. Instead multiple different signals must be assessed and this requires the use of a network where multiple sensors work together to detect multiple different signals and to make the correct decision (a multikinase-network; Figure 1B). In the era of ever- increasing antibiotic resistance, these networks make attractive potential drug targets as they are absent from humans and are needed for bacterial survival and virulence. However, a major roadblock in researching these networks is determining which sensors participate in them. We have developed a bioinformatic method that allows us to predict these sophisticated networks and will therefore streamline the process of discovering these promising drug targets.

This project is focussed on a unique multikinase-network that we have predicted in the melioidosis pathogen, Burkholderia pseudomallei, which comprises two sensor kinases that are essential for cell growth. It is likely to be an excellent target for the development of antimicrobial drugs. Research Objectives:

1. Show that the sensors work together as a network using interaction assays (two-hybrid and phosphorylation assays).

2. Determine what this network controls and why it is essential.

3. Identify the stimuli sensed by the kinases using binding assays and structural characterisation of the sensory domains using X-ray crystallography.

Achieving these objectives will, firstly, characterise a crucial multikinase-network in an important antibiotic resistant pathogen and secondly, by validating our method for predicting multikinase-networks will greatly accelerate the discovery of other examples of these promising potential drug targets.

Rotations: 1. Steve Porter and Rick Titball: Predicting multikinase-networks in Burkholderia pseudomallei. Skills gained: bioinformatic analysis of specificity residues, molecular biology and

protein-protein interaction assays. 2. Ravi Acharya: Structural biology of the essential kinases in Burkholderia pseudomallei. Skills gained: protein purification and X-ray crystallography.

Training potential: The student will develop a broad range of interdisciplinary skills important for systems biology research including: molecular microbiology, protein biochemistry, structural biology, bioinformatics, sequence analysis and molecular biology.

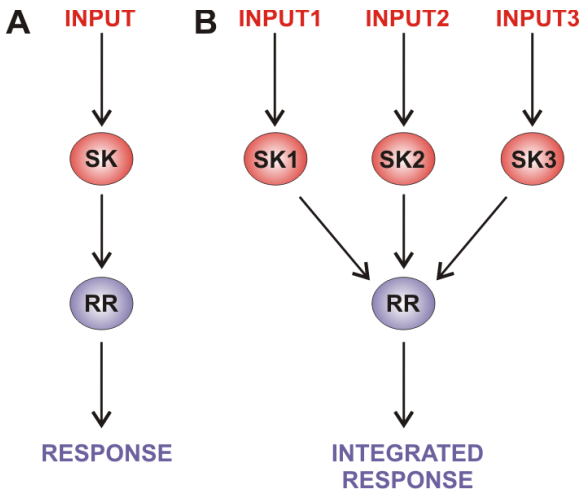

Figure 1: Multikinase -networks. A) A simple two-component system comprising a single sensor kinase (SK) that signals to a single response regulator (RR). B) A multikinase network comprising three SKs controlling a single RR.