Title:

A kinetic analysis of mycobacterial two component system (TCS) using High Throughput Assay (HTA), mathematical modelling and Microscale Thermophoresis (MST)

Abstract:

Two component signaling systems (TCSs) are central to bacterial adaptation and present promising drug targets. The mechanisms underlying most of the reactions involving bacterial TCS proteins and their reaction rates are undetermined on account of technical challenges associated with the chemistries of the phosphorylated proteins. Here, we quantified kinetic parameters of sensor kinase (SK) autophosphorylation a rapid high throughput assay (HTA) platform. We investigated the autophosphorylation kinetics of three promiscuous SKs of *M. tuberculosis* We developed a mathematical model based on the experimental observations and model predictions were in agreement with the experimental data. Best-fit parameter values yielded estimates of the extent of SK-ATP association and the rates of SK autophosphorylation, allowing for the first time a possible rank ordering of the SKs in terms of the efficacy of their autophosphorylation reactions. MtrB emerged as the fastest autophosphorylating and interestingly is the most promiscuous SK. We quantified apparent binding affinities of autophosphorylated MtrB (MtrB~P) with its cognate and the non-cognate response regulators (RR) using Microscale Thermophoresis (MST) technique. This revealed the relative propensities of phosphotransfer of MtrB~P to other non-cognate RRs. Surprisingly, the cognate RR, MtrA has about a log fold lower affinity towards MtrB~P than the other non-cognate RRs studied. This finding reinforces a study which suggests MtrA phosphorylation is avoided by dispersion of autophosphorylated MtrB. Since binding of phosphorylated SK to RR precedes the phosphotransfer event to RR, the relative binding affinities inform us about the most probable crosstalk links. We, therefore, obtained binding affinities of most of the cognate and non-cognate interactions Such quantification may, present insights into the design principles governing TCS signaling as well as those underlying survival and adaptation of bacteria brought out by mathematical modelling using kinetic parameters. Overall, the HTA platform together with the model provides a facile tool to quantify reactions involving bacterial TCS proteins and MST provides the relative propensities of SK towards various RRs, useful in elucidating features of dynamic TCS network.