Project 1. Structure-guided discovery of allosteric modulators of kinases

Most known kinase inhibitors and therapeutics are Type 1 agents that target the ATP binding site. Due to the highly conserved ATP binding pocket, these competitive inhibitors have less than ideal selectivity, which contributes to their toxicity. Allosteric modulators (antagonists and agonists) have greater potential than ATP-competitive kinase inhibitors to achieve selectivity and potency since such modulators target binding sites that are less conserved within the kinases and even among closely related isoforms. Allosteric modulators can also regulate enzyme functions in untraditional ways because the mechanism of action is totally different from the conventional ATP-competitive inhibitors. Thus, allosteric antagonists and agonists can be applied to investigate specific biological and pathological functions of kinases in human diseases, advancing biomedical research and therapeutic discovery. Unfortunately, very few allosteric modulators of kinase have been discovered, and none of them have been developed as therapeutic agents. Discovering allosteric modulators is a challenging task since it is difficult to define allosteric binding sites and identify potential starting chemical agents.

In our pilot studies, we designed and synthesized allosteric modulators of kinase by applying structure analysis and computer-aided molecular design techniques. As shown in Fig 1, peptidomimetics 31 and 35 enhance enzyme activity upto 350% at 10 μM, which confirms that these are allosteric agonists of kinase. In order to develop allosteric agonists of kinase, cyclic peptidomimetics were designed and synthesized. As shown in Fig 2A, these peptidomimetics inhibit the kinase activity upto 90% (i.e. 10% enzyme activity in the presence of Pep56) at 50 μM. Confirmation of this result is provided by the concentration-dependent inhibition of kinase by pep53 and pep56 (IC_{50} = 25 ~ 50 μM, Fig 2B).

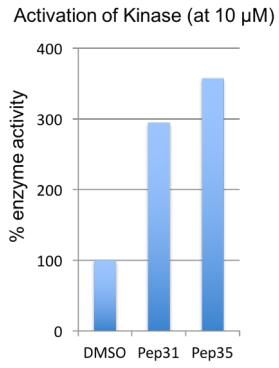

Figure 1. Allosteric agonists of kinase

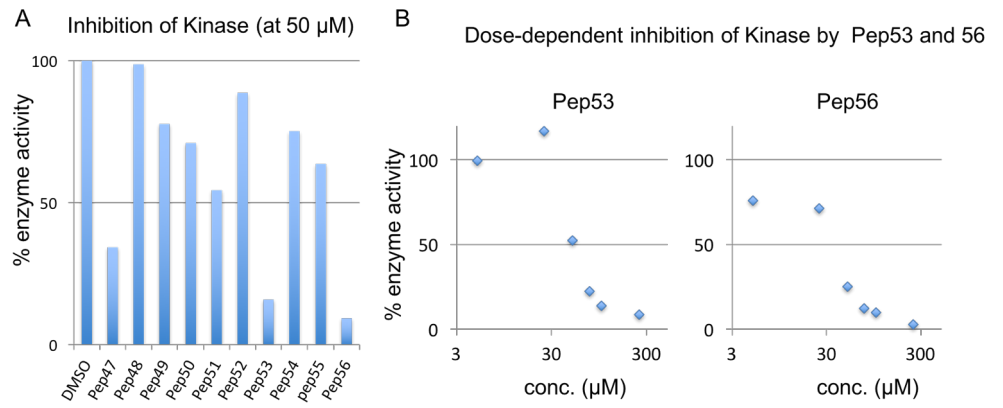

Figure 2. Allosteric antagonists of kinase

The pilot study confirmed our hypothesis, and we were able to determine an allosteric pocket of kinase and identify starting chemical agents. Our in-house molecular models of kinase in complex with peptidomimetics clearly explain their agonistic and antagonistic activities. Therefore, these peptidomimetics are used as starting chemical agents for structure-guided hit-to-lead optimization for the development of allosteric agonists and antagonists. Furthermore, computational models of kinase - modulator complex are utilized for the design of small molecule agonists and antagonists of kinase. The allosteric modulators developed in our laboratory will be new entities that will be utilized as highly specific chemical probes to elucidate allosteric mechanisms, unique biological functions, and pharmacological roles of kinase. Once we develop highly potent allosteric modulators of kinase, these agents will be applied to investigate specific role of kinase in cells and *in vivo* models of human diseases.