DESIGN OF BIOSWITCHES FOR SYNTHETIC BIOLOGY

An-Ping Zeng, Hamburg University of Technology, Germany
aze@tu-harburg.de
Chengwei Ma, Hamburg University of Technology, Germany

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Novel bioswitches are of great interest for synthetic biology, especially when dynamic control of metabolic fluxes is demanded. Among the natural bioswitches, riboswitches and allosteric proteins are of particular importance because of their wide distributions in nature. However, the application of natural bioswitches is often limited by their narrow response range and the engineering of allosteric proteins is challenging due to their dynamic feature and complex mechanisms, especially for non-natural ligands. In this presentation, first an efficient approach that is able to extend the ligand response range of riboswitches will be presented by taking advantage of the computer-aided rational design. A lysine riboswitch from E. coli I has been employed as a model system to demonstrate the procedure. Then, a novel strategy to explore and engineer ligand-induced allosteric regulation based on a new concept of thermodynamic model of protein conformational dynamics will be presented with the aim to create allosteric regulations for non-natural ligands. The key feature of the thermodynamic model is that the allosteric process upon ligand binding is divided into two sub-processes – conformational change and molecular binding. As a consequence, the ligand-induced allosteric regulation can be explored for each sub-process from both energetic and structural perspectives. To prove the concept, aspartokinase III from E. coli was used as a model system. Guided by the thermodynamic model, the natural ligand has been successfully altered from an inhibitor to an activator. Moreover, both inhibition and activation effects have been established for a non-natural ligand.