Insights on the ‘Venus flytrap mechanism’: solution structure and segmental motion of periplasmic binding proteins

David Castaño, Gabriel Ortega, Oscar Millet

Structural Biology Unit, CICbioGUNE, Bizkaia Technology Park Bld. 800, 48160 Derio, Spain. omillet@cicbiogune.es

Conformational flexibility is an intrinsic property of proteins, often used by nature as a tool in the biological regulation of processes at a molecular level. For instance, a change in the conformation of a protein after binding a ligand may create a new binding site that may result in the activation of a signal transduction pathway. Identification of the specific interactions that govern the thermodynamic equilibria between the different conformations becomes crucial to understand these cellular mechanisms. Unfortunately, a detailed analysis of the high resolution structure of the complex is not enough, due to the fact that subtle interactions, even far away from the binding site, may play a key role in the stabilization of a given conformation. With the final goal of understanding such driving forces, it is desirable the development of methods that allow evaluating the energy associated in a segmental reorientation in an analogous way to the measurements of the molecular stretching tension in atomic force microscopy experiments.

In this project we show how NMR experiments, in combination with site directed mutagenesis can be successfully applied to investigate the 'Venus flytrap mechanism' that occurs in the super-family of bacterial periplasmic receptors. Residual dipolar couplings have been used to determine the average inter-domain orientation in solution, while accurate relaxation measurements were used to estimate the energetic (entropic) contribution. The integration of all the information has resulted in a structural model for the closure mechanism that is being validated with the use of mutations.