iNEXT workshop on Integrated methodologies and approaches for structural biology

Name of Speaker: **Roland Riek**
University / Research Institute / Department: **ETH/ D-CHAB**

Title of Lecture: **Protein Allostery at atomic resolution**

Abstract:

Allostery in proteins describes the process by which an effect such as ligand binding on one site of a protein or protein complex is transmitted to another distal functional site regulating thereby biological activities. Several models on the mechanism of allostery have been postulated including the sequential mechanism, the conformational selection mechanism and the dynamic allostery model. However, the elucidation of the nature of this 'action at a distance' phenomenon appears to be rather difficult and is gathered rather a "biophysical enigma eluding a general, quantifiable and predictive atomic description" because either only low resolution, local data are available (which includes relaxation studies by NMR) or individual constrained structures were determined (such as X-ray structures free and in complex of ligands, transition intermediates, etc) eventually combined with molecular dynamic simulations using recently developed sophisticated statistical analysis.

Here, we present three methods for the study of protein allostery: (i) multi-state structure determination using exact NOEs (Vögeli et al., 2012) revealing the diverse interconverting structural states of a protein at atomic resolution, (ii) a novel analysis of protein-ligand NMR titration experiments that reveal quantitatively the equilibrium constants and energies of the allosteric coupling through the protein at residue resolution and (iii) a polychromatic pulse-based CEST NMR experiment, which moves CEST NMR from the CW mode to the FT mode enhancing the signal to noise accordingly.

With these methods the allostery of the WW domain of the proline cis/trans isomerase Pin1 with a coupling between the ligand binding site and the inter-domain interaction site was studied. It is found that in the absence of ligands, the protein undergoes a micro-second exchange between two states, of which one is beliefed to be fit to interact with the catalytic domain, while the other one is not. In presence of the positive allosteric ligand, the equilibrium between the two states is shifted highlighting the mode of ligand action to be conformational selection as proposed by Monod. In contrast, the allostery-surpressing ligand is decoupling the side chain arrangement at the inter phase into an anti-correlative orientation and dynamics thereby reducing the inter-domain interaction. Its action can thereby regarded to be of dynamic allostery nature. The presented distinct modes of action highlight the power of the dynamics-structural interplay in the biological activity of proteins.

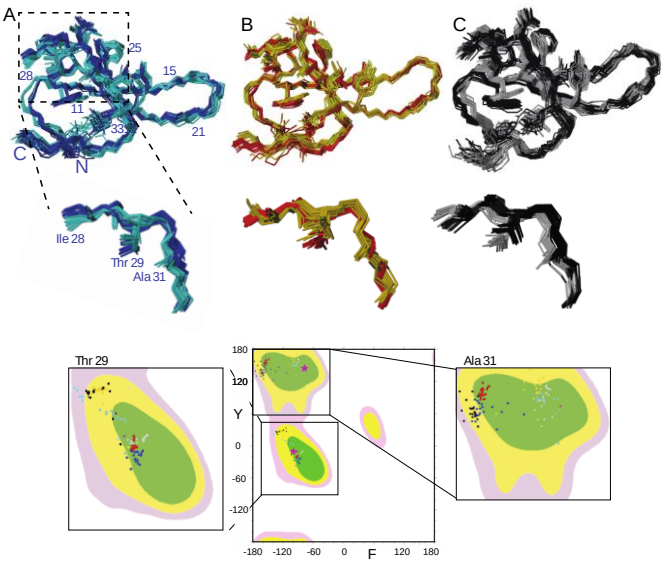

Figure: Two state structural ensemble of (A) apo WW, WW in complex with the positive (B) and negative (C) allosteric ligands highlighting the presence of two distinct states. Backbone trace of 20 structural ensembles of the WW domain each representing two different states are indicated. In addition severa side chains are shown and labeled. The WW states were color coded with cyan and blue for the apo WW, yellow and red for the positive allosteric ligand FFpSPR complex, and grey and black for the negative allosteric ligand pCdc25C complex, respectively. The two states of the catalytic-domain interacting Loop 2 are enlarged as indicated. In addition the Ramachandran plot for Ile 28, Thr 29 and Al31 are shown with the same color code as in the structures. The Ramachandran angle of the x-ray structure is shown with a pink star.

Research Profile:

The structural biology of protein aggregation, the multi-dimensional structure/dynamics activity relationship of proteins and the interplay between membranes and proteins are the foci of our research using nuclear magnetic resonance spectroscopy as the main technique.

Three selected publications:

1. Vögeli B, Kazemi S, Güntert P, Riek R: **Spatial elucidation of motion in proteins by ensemble-based structure calculation using exact NOEs**. *Nat Struct Mol Biol* 2012, **19**(10):1053-1057.
2. Vögeli, B., Segawa, T.F., Leitz, D., Sobol, A., Choutko, A., Trzesniak, D., van Gunsteren, W., Riek, R. (2009) **Exact Distances and Internal Dynamics of Perdeuterated Ubiquitin from NOE Buildups**. *J. Am. Chem. Soc.* **131**, 17215–17225.
3. Chi, CN., Vögeli, B., Bibow, S., Strotz, D., Orts, J., Güntert, P., Riek, R. (2015) **A Structural Ensemble for the Enzyme Cyclophilin Reveals an Orchestrated Mode of Action at Atomic Resolution**. *Angew Chem Int Ed*. **54**, 11657-11661

CEITEC Masaryk University
Kamenice 735/5
625 00 Brno, Czech Republic
+420 549 495 511, info@ceitec.cz
www.ceitec.eu

CEITEC Masaryk University
Kamenice 735/5
625 00 Brno, Czech Republic
+420 549 495 511, info@ceitec.cz
www.ceitec.eu