Structural Basis for the Recognition of the Proline Rich Sequences by FBP-21 tandem-WW domains

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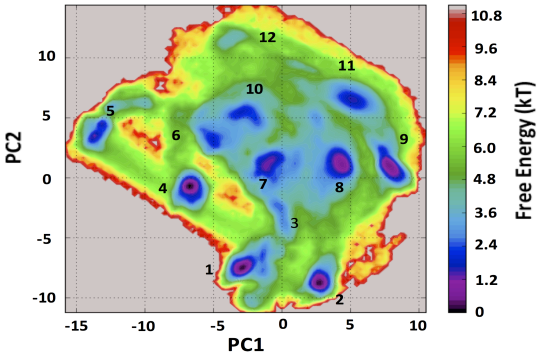

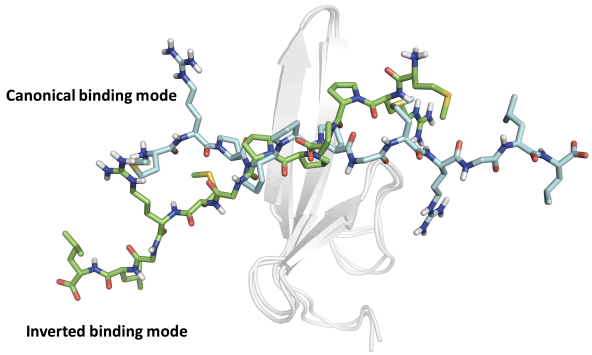

Formin-binding protein 21 (FBP-21) is a spliceosomal protein, which recognizes the proline-rich sequences (PRS) abundant in various splicing factors. The recruitment of PRS by tandem-WW domains (t-WW) of the FBP-21 is characterized by the low affinity, which in turn can be enhanced by the multivalent binding. Still the binding of the multivalent PRS to the t-WW is poorly understood. [1] In this study, we aim to elucidate the structural determinants of a such multivalent recognition by combining the nuclear magnetic resonance (NMR), isothermal titration calorimetry (ITC), molecular dynamics (MD) simulations, and protein-peptide docking.

NMR, and ITC measurements revealed that the monovalent PRS bind with higher affinity to a singular WW_1 construct compared to the t-WW domains. This finding raised a question, if there is a structure in the conformational ensemble of the apo t-WW domains, which favors binding to the WW_1 domain. We performed PCA analysis on the MD simulations of the apo t-WW domains, and showed that there is a structural preference for the higher WW_1 domain affinity. There are 12 long-lived structures in the conformational space of the apo t-WW domains, determined by the cluster-specific hydrogen bond network. Only in a single cluster, residues of the WW_1 domain binding groove are involved in the inter-domain interface formation. On contrary, in five clusters, the interface formation hinders the WW_2 domain binding groove residues from binding. Other six clusters are free to bind PRS in both binding grooves. However, further structural rearrangement is necessary, so that the relative orientation of the respective binding grooves is proper for the multivalent recognition.

As it was earlier confirmed by the spin labelling experiments [1], PRS can be docked to the t-WW domains in a canonical, or in an inverted binding mode. We ran the protein-peptide docking calculations, and found the same binding behavior. Both binding complexes were stable during the 20 ns MD runs, and serve as a basis for the modeling of the multivalent recognition by t-WW domains.

In conclusion, current efforts in our groups are towards proving a hypothesis, that multivalent recognition of the PRS by t-WW domains is governed by an allosteric mechanism. This mechanism comprises the initial recognition of the multivalent PRS by the WW_1 domain, triggering the structural rearrangement of the flexible linker, and bringing the WW_2 domain in the proper orientation to bind the second valance of the respective multivalent PRS.

[1] S. Klippel *et. al*, *J. Bio. Chem.*, **2011**, *Vol. 286*, 38478-38487.