A New look on Induced Selectivity in large IDP's and its complexes: NMR-based characterization and biological function of CD2AP bivalent binding to intrinsically disordered domain of chikungunya virus nsP3 protein

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Life science latest discovery shows the fundamental significance of intrinsically disordered proteins (IDP) particularly in the functionality of eukaryotes. The key challenge is how to link the disordered state of IDP with events leading to complex formation with host proteins and rationalise this process from the fundamental physico-chemical basis of the conformational behaviour of single molecules. Even though the number of this type of studies escalates rapidly the nature of the selective interaction in IDPs is unknown. This is mostly due to the reason that up till now results obtained on **small sized** IDP models by traditional X-ray/NMR studies often seems to be non-applicable or even misleading to the **larger biologically** relevant models of IDPs.

Here we present the result of a study[1,2] of the alphavirus non-structural protein nsP3 containing a long, disordered, hypervariable domain, HVD, that determines viral adaptation to different cells. We applied new acquisition and assignment approaches in NMR spectroscopy to characterize interaction of chikungunya virus (CHIKV) nsP3 HVD with SH3 domains of one of the critical, cellular, HVD-binding partner CD2AP. The data demonstrate that CHIKV HVD contains both canonical and non-canonical SH3 domain-binding sites. Their cooperative interaction with two SH3 domains of CD2AP increases affinity of protein binding and induces long-range conformational changes in HVD. Point mutations in both binding sites abolished HVD interaction with CD2AP and two other SH3 domain-containing cellular proteins. These mutations had strong negative effect on the early step of viral replication in human cells, and demonstrated that HVD binding to SH3 domains plays a critical role in the formation of viral replication complexes.

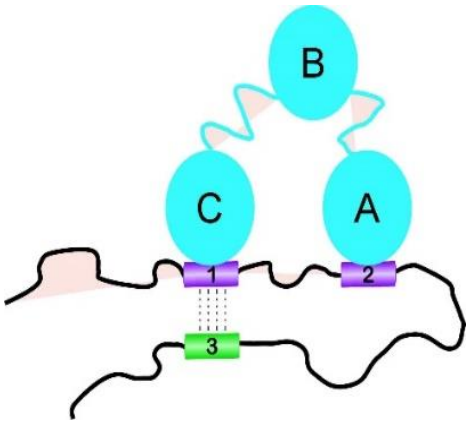

Figure 1 Schematic model of CHIKV HVD complex with CD2AP.

[1] T.A. Agback & P.Agback et al. *J. Virol.* **2018**, *92(16)*, e00838-18
[1] P.Agback &T.A. Agback et al. *NatureResComm*, **2019**, *submitted*