ABSTRACT

To the PhD thesis titled

"Investigation of the dynamic basis of protein-protein and protein-DNA interactions by the intrinsically disordered region of the HOX transcription factor Sex Combs Reduced by solution NMR spectroscopy"

by

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Eukaryotic proteins have long stretches of amino acids, which do not fold into any three-dimensional structure. These intrinsically disordered regions (IDRs) interact with multiple macromolecules and often act as hubs in protein interaction networks (PINs). IDRs contain short linear motifs (SLiMs) of 3 to 10 residues, which are sites for interaction with other macromolecules and post-translational modifications. However, SLiMs are hard to identify by experimental or bioinformatics methods. Till date only ~4000 SliMs have been identified out of possible 1,000,000 SliMs in the human genome. This work investigates the physicochemical basis of function of SliMs and the possibility of their identification by NMR dynamics experiments.

The picosecond-nanosecond timescale backbone dynamics of a *Drosophila* HOX transcription factor sex combs reduced (SCR) revealed that the residue-wise flexibility significantly varied in its IDR. Short stretches (~5-7) of relatively rigid amino acids were identified, which were linked by highly flexible amino acids. Moreover, one of the identified rigid segments interacted with high specificity to a partner transcription factor extradenticle. Overall, this work provides clear experimental evidence for the "rigid segment model" of IDRs where the rigid segments facilitate interaction with cofactors by dampening the loss in conformational entropy upon binding. Also, NMR dynamics provides a powerful means to identify rigid segments in IDRs, which can potentially be functional SLiMs.

This work also shows that the NMR relaxation parameters R_1, R_2 and NOE can be combined as $R_1R_2/(1\text{-NOE})$ to efficiently measure residue-wise rigidity of IDRs. The robustness of $R_1R_2/(1\text{-NOE})$ in measuring residue-wise rigidity as compared to the order parameter (S^2) was tested. Both identify the same rigid and flexible segments in the IDR, although $R_1R_2/(1\text{-NOE})$ has a greater dynamic range compared to S^2. Furthermore, mutants were designed to alter the flexibility of the linker region in SCR, and both methods showed that the linker becomes less flexible. However, no change in the ensemble properties were observed for these mutants. Interestingly, these mutants had weaker DNA-binding affinity compared to the wildtype protein. Thus, residue-wise rigidity plays an important role in IDR function and it can be measured using the straightforward and robust parameter $R_1R_2/(1\text{-NOE})$.

Keywords: Short linear motifs (SLiMs), rigid segment model, electrophoretic mobility shift assay (EMSA), fast-timescale dynamic, order parameter (S^2), molecular dynamics (MD) simulation, analytical size exclusion chromatography (SEC)