Molecular interactions of poly(PR) dipeptides in liquid-liquid phase separation

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Arginine-rich dipeptides repeat proteins (DPRs) encoded by a hexanucleotide expansion in C9ORF72 gene are known to be responsible for neurodegeneration in amyotrophic lateral sclerosis (ALS) [1, 2, 3]. These DRPs, along with RNA and other proteins, form liquid-liquid phase separation (LLPS) in cells, and impede functions of membrane-less organelles including nucleoli [4, 5], depending on their sequence [poly(PR) and poly(GR)]. However, the correlation between the primary peptide sequence and toxicity remains unclear.

In this research, we performed Molecular Dynamics (MD) simulations, Fluorescence Recovery After Photobleaching (FRAP) and proteomic experiments to understand the molecular interactions of DRPs in the LLPS, focusing on understanding of the three body interactions between DRPs, RNA, and other proteins. Firstly, we estimated the binding affinity between RNA and poly(PR) sequence variants by MD calculation shown in Fig. 1. We designed three peptides with different charge distributions arising from their primary sequence, where Proline (P) and Arginine (R) exist with a unique periodicity. It is found that their binding affinity depends on the primary sequence of peptide due to the contribution of electrostatic force. Solvation of DRPs is also controlled by the sequence, and water molecules distribute separately or repeatedly due to the presence of P and R. Rigidity of the peptide was found to be regulated by prolines. Next, we performed cell-based proteomic analyses. The results revealed that DRPs can efficiently trap proteins with acidic stretches, such as a nucleolar protein, NPM1. Lastly, we conducted FRAP experiments, which provide us diffusion coefficients of DRPs, RNA, and NPM1 in LLPS. While diffusion coefficients obtained from the FRAP showed a good correlation with the MD results in the case of two body interactions of DRPs vs RNA, diffusion coefficients of proteins in LLPS of living cell showed unique behavior, which implied a necessary to take into account three body interactions between DRPs, RNA, and other proteins such as NPM1.

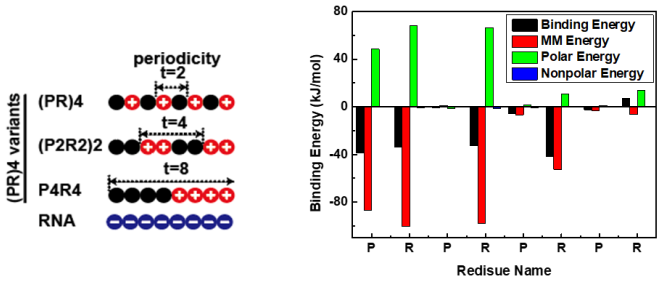

Fig.1. Charge distribution of each peptides (left) and the binding energy and its contribution components of $(PR)_4$ variants

Reference:

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