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題目(和文)	PR繰り返し単位を持つポリペプチドとRNAによるLLPS液滴の構成及び固液界面における拡散の分子理解
Title(English)	Molecular understanding on LLPS droplets consisting of poly(PR) dipeptide repeats and RNA and its diffusion at solid/liquid interface
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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	有機・高分子物質　系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　工学　）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In the present thesis, to understand the process of Arg-rich dipeptide repeat proteins (DPRs) to form liquid-liquid phase separation (LLPS) and its wetting property, which have close correlation to its biological function and even neurodegenerative diseases, like ALS, the molecular interactions of DPRs in the LLPS and also the LLPS droplets diffusion at solid/liquid interface in a dynamic manner have been investigated.

In chapter 2, we designed a series of poly(PR) variants: $(PR)_4$, $(P_2R_2)_2$ and P_4R_4 with the same total length and density of positive residue Arg but different periodicities (the size of periodicity t = 2, 4 and 8) and homopolymeric adenine poly-rA as a model for RNA. Then, we utilized molecular dynamics (MD) simulations on one-by-one interactions, which can provide detailed information on the conformational changes and thermodynamics of biological molecules and their complexes at atomic level. As a result, we found the binding energy strength was inversely proportional to the periodicity value of poly(PR) variants. Alternating charge distribution in poly(PR) results in lower binding energy while poly(PR) variants with higher periodicities (e.g. PnRn) have higher binding energy when interacting with RNA. Besides, we also calculated free energy landscape for the interacting complexes in order to explore Pro residues contribution towards structural property of poly(PR) variants. We found that Pro residues not only modulated solvation of water on the biomolecular surface but could confer rigidity for peptide structures and thus intervened the interaction between cationic amino acids and anionic nucleic acids.

In chapter 3, we *in vitro* and *in vivo* experimentally demonstrated the relationship between Arg distribution of poly(PR) variants and their interaction with RNA and

protein. We firstly synthesized poly(PR) variants: $(PR)_{12}$, $(P_2R_2)_6$, $(P_4R_4)_3$ and $P_{12}R_{12}$ and poly-rA as RNA. Droplets of $(PR)_{12}$ variants with larger periodicities lost their spherical structure under this condition, and $P_{12}R_{12}$ formed sticky non-spherical condensates losing surface tension minimization as the typical characteristic of liquids. Next, we conducted fluorescence recovery after photobleaching (FRAP) experiments to investigate the dynamic diffusion process of poly(PR) variants LLPS droplets from the perspective of two-body interaction (poly(PR) variants and RNA) and three-body interaction (poly(PR) variants, RNA and protein). For two-body interaction, we found RNA mixed with $(PR)_{12}$ had a higher fluidity than RNA mixed with $(P_4R_4)_3$ or $P_{12}R_{12}$ and also Pro residue could loosen complex coacervation structure, which provide enough multivalency to form LLPS droplet according to critical salt concentration (CSC) experiments. For three-body interaction, we observed that the rate of dynamic exchange of NPM1 protein in phase equilibrium was disrupted by complex coacervation with $(PR)_n$. $(PR)_n$ condensates NPM1 protein and rRNA in phase-separated droplets via multivalent interaction, thereby affecting the mobility of NPM1 protein in cells, whereas P_nR_n failed to condense NPM1 protein and accumulated to rRNA. Thus, P_nR_n did not significantly affect NPM1 protein mobility.

In chapter 4, we did cell-based proteomic analysis to explore the interaction motif of protein for poly(PR) peptide. We found that dipeptide-protein interaction was affected by the size of $(PR)_{12}$ periodicity and alternate Arg structure of DPRs could efficiently enhance dipeptide-protein interaction. Next, we did structural decoding for these proteins and demonstrated the electrostatic interactions between positive Arg amino acids and acidic motifs were the main driving force for interactome enrichments, which could be explained, at least partially, by LLPS.

In chapter 5, we focused on the dynamic diffusion process of LLPS droplets consisting of ploy(PR) dipeptide repeats, fluorescence tagged poly(PR) dipeptide repeats and poly-rA RNA at solid/liquid interface. We explored two solid surfaces, one was untreated cover glass and the other was positive APTMS chemically

modified cover glass, and then utilized fluorescence microscope to observe the diffusion of LLPS droplets on these two solid surfaces, respectively. As the result, after using single particle analysis, we found two diffusion modes, fix mode and diffusion mode. The chemical modification of the glass surface by amine termination tuned the distribution of these diffusion modes. Meanwhile, the negative zeta potential of LLPS droplet surface probably tended to increase its interaction with positively charged glass surface, resulting in exhibiting more droplets in the fix mode. These results could provide an improved method to advance fundamental studies for understanding nature of LLPS droplets.

Investigation on the molecular interactions of poly(PR) dipeptide repeats in the LLPS and the droplets diffusion at solid/liquid interface, we found the alternate distribution of Arg and Pro in poly(PR) controls for phase separation of poly(PR) with proteins /nucleic acids and determines NPM1 protein mobility, which may be helpful to explain the impedance of functions of membrane-less organelles including nucleoli and ALS disease pathology. Besides, we also focused on the LLPS diffusion modes at two different solid surfaces in aqueous environment, one is untreated cover glass and the other is chemically modified cover glass with amine groups, which can provide an improved method to control LLPS diffusion at solid/liquid interface by engineering methods to tune LLPS coalescence and fusion. Most importantly, the study of LLPS interface interaction with outer medium is closely related to LLPS uptake for surrounding molecules. The control of this uptake process is desired to be helpful for the curation of ALS disease in the near future.