



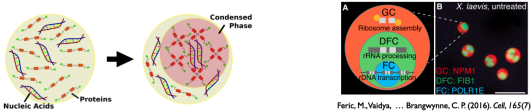
Sequence dependent phase separation of protein-polynucleotide mixtures

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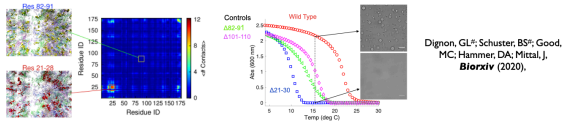
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Introduction

- Biomolecules (protein and RNA) co-phase separate to form Membraneless Organelles like Ribonucleoprotein (RNP) granules



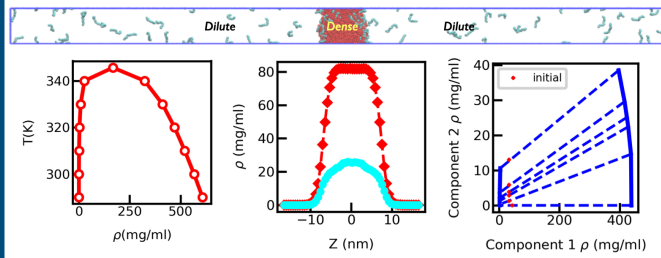
- Our Coarse-Grained model is able to capture and predict sequence-specific phase separation of IDPs



- We extend our CG modeling framework to model RNA-protein interactions which could drive the formation of RNP granules

Coexistence simulation method

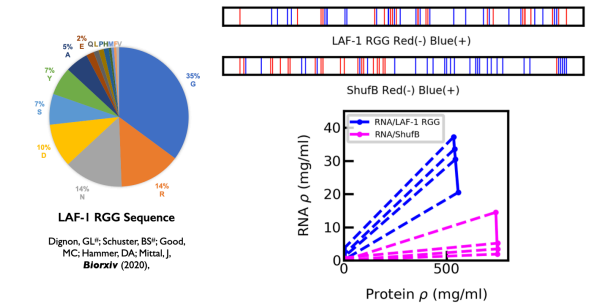
- Simulations in a slab geometry allow for efficient sampling of coexisting phases



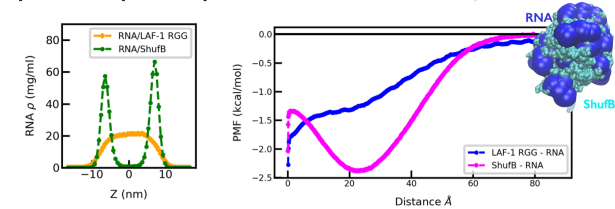
- Densities of RNA and protein in the dilute and dense phases are used to construct single and multicomponent phase diagrams
- Multicomponent phase diagrams quantify co-phase separation

Role of protein sequence

- Charged amino acids of LAF-1 RGG were shuffled to create a charge patterned sequence (ShufB)



- Multicomponent phase diagram shows substantial shift in RNA-protein co-phase separation behavior in the ShufB case

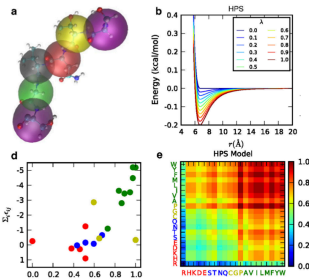


- RNA doesn't mix well in the ShufB condensed phase as compared to LAF-1 RGG
- Condensed phases with complex microstructures can be formed which can be explained by protein-RNA PMFs

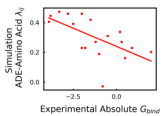
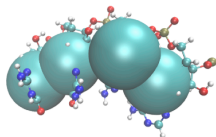
Computational models

CG model for IDPs

- Single bead per residue
- Uses an atomistic hydrophathy scale or a bioinformatics based contact potential (M) to describe short range interactions between amino acid pairs



Dignon, G. L., ... Mittal, J. (2018). *PLoS Computational Biology*, 14(1), e1005941.

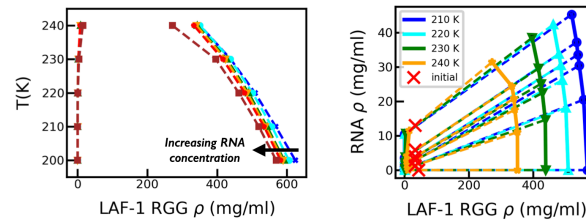


De Ruiter, A. & Zagrovic, B. *Nucleic Acids Res.* 43, 708-718 (2015).

CG model for RNA

- Single bead per nucleotide
- Each bead given unit negative charge
- Short-range interactions between RNA-RNA and RNA-protein beads are parameterized using hydrophathy values similar to the protein model

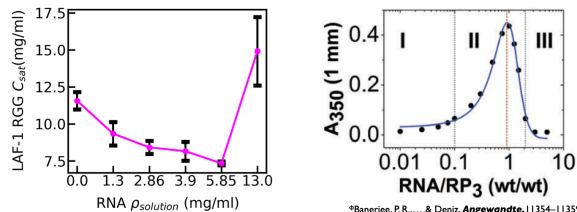
Effect of RNA on protein LLPS



- Multicomponent mixture shows Scaffold (LAF-1 RGG) - Client* (RNA) co-phase separation
- LAF-1 RGG phase diagram shifts leftward on adding RNA in agreement with experiments from Brangwynne# and others

*I. Lin, Y. H. Brady, J. P. Forman-Kay, J. D. & Chan, *New J. Phys.* 19, (2017).

#I. Wei, M. T. et al. *Nat. Chem.* 9, (2017).



*Banerjee, P. R., ... & Deniz, *Angewandte*. 11354-11359

- Reentrant phase transition observed with increasing RNA concentration which is in agreement with experiments^Φ

Conclusions

- LAF-1 RGG/RNA mixture undergoes Scaffold (LAF-1 RGG) — Client (RNA) co-phase separation
- Adding RNA initially facilitates phase separation but after a point the system undergoes a reentrant phase transition
- Shuffling the protein sequence reduces the RNA incorporation drastically hence revealing the sequence dependence of co-phase separation behavior

Funding: National Institutes of Health (NIH) [R01GM136917]