Amino acid sequence and RNA secondary structure content effect on emergent material properties of condensates

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G-quadruplex (rG4) is a non-canonical RNA secondary structures made of guanine rich RNA sequences via the stacking of Hoogstein-bonded guanine quartets. rG4s structures can undergo phase separation by themselves or when paired with anionic polypeptides through multi-valent interactions. An increase in RNA rG4 content results in the formation of 'solid-like' aggregates when combined with poly-lysine (pK) peptide sequences.[1]

Fragile X Mental Retardation Protein (FMRP) is an RNA binding protein that can undergo phase separation in the presence of RNA and targets rG4 rich sequences [2]. FMRP contains arginine RGG rich domain in its low complexity region with high specificity to rG4 structures. Thus, questions arise regarding the mechanisms surrounding liquid-like phase separation as it pertains to rG4 content and the role that rG4 plays when interacting with different types of amino acid sequences.

Here, we investigate the emergent material properties of condensates made of different peptides and proteins as they interact with RNA with increasing rG4 content. We also investigate how these condensates behave when incubated together in competitive, limiting RNA circumstances.

We find that, in the presence of Map1-b (an FMRP RNA target with high G-quadruplex content), pK and poly-arginine (pR) result in the formation of aggregates with distinct physical properties. Aggregates made of pR-Map1b melt at a higher temperature than those of pK-Map1b. Furthermore, these aggregates present distinct morphologies depending on polymer identity. Finally, incubating FMRP, pK and Map1b together results in different outcomes when one varies the order of addition.

Although, for certain peptides, droplets can be tuned via rG4 content modulation, the effect of rG4 is nuanced and seems to depend on the sequence of the peptide or protein. It remains to be investigated the mechanical role that FMRP, pK and pR play which distinguishes their distinct effects on droplet and aggregate formation.

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(2) Tsang, B.; Arsenault, J.; Vernon, R. M.; Lin, H.; Sonenberg, N.; Wang, L.-Y.; Bah, A.; Forman-Kay, J. D. Phosphoregulated FMRP Phase Separation Models Activity-Dependent Translation through Bidirectional Control of MRNA Granule Formation. *Proceedings of the National Academy of Sciences* **2019**, *116* (10), 4218–4227. https://doi.org/10.1073/pnas.1814385116.