Building artificial RNA organelles via phase separation

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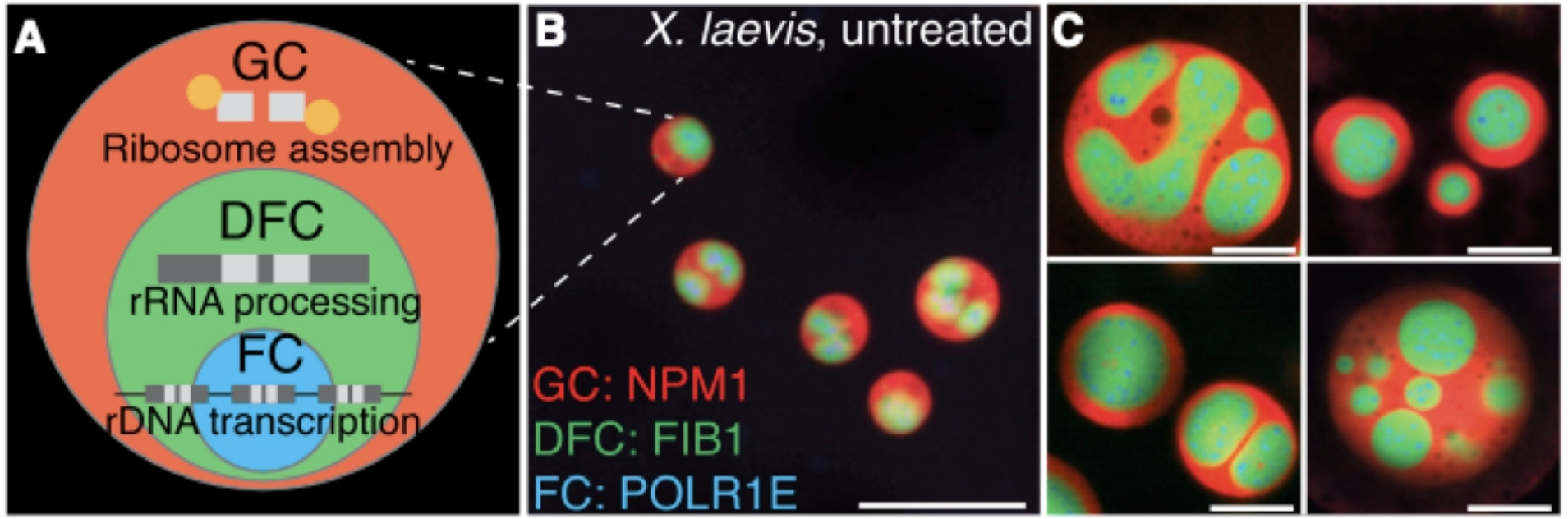
Why phase separation?

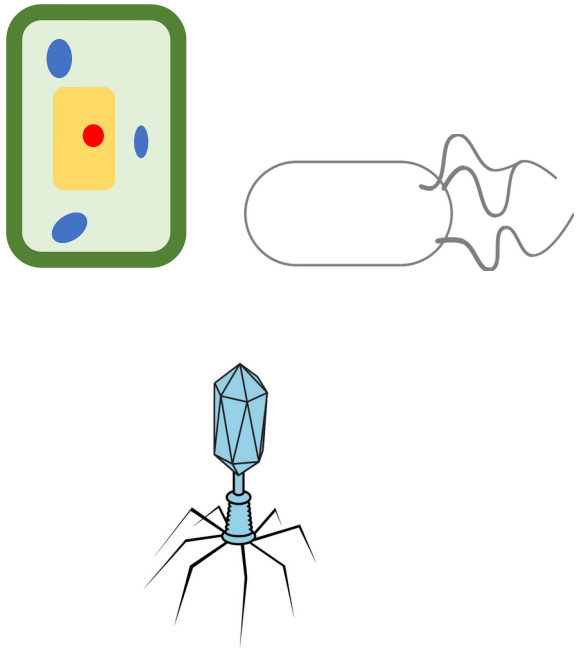
- No membrane required
- Adaptive, dynamic compartments
- Phase separated organelles exist in nature (nucleolus, stress granules)



The nucleolus (ribosome biogenesis) is a phase separated organelle. Feric et al. Cell 2019

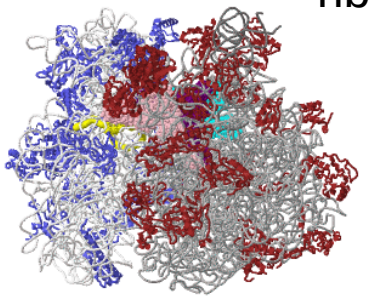
Why RNA?

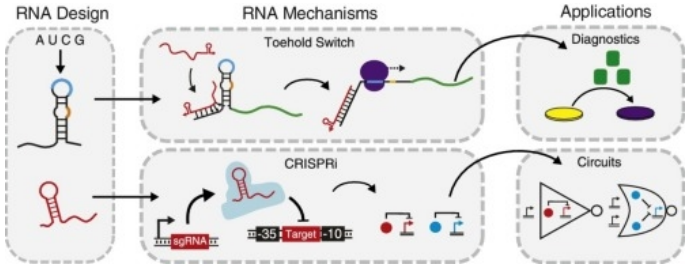
- Naturally present in all forms of life



- Multifunctional
- Store information
- Structural element

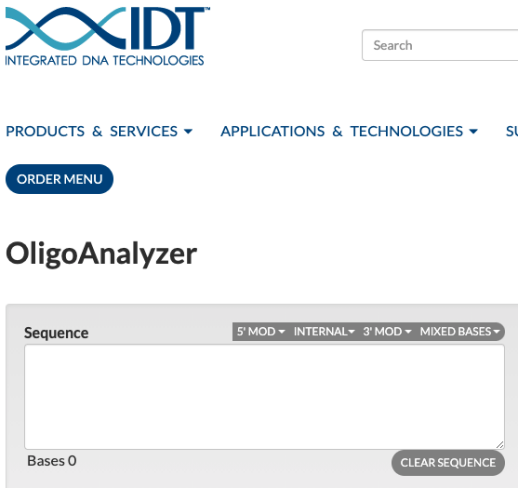
ribosome



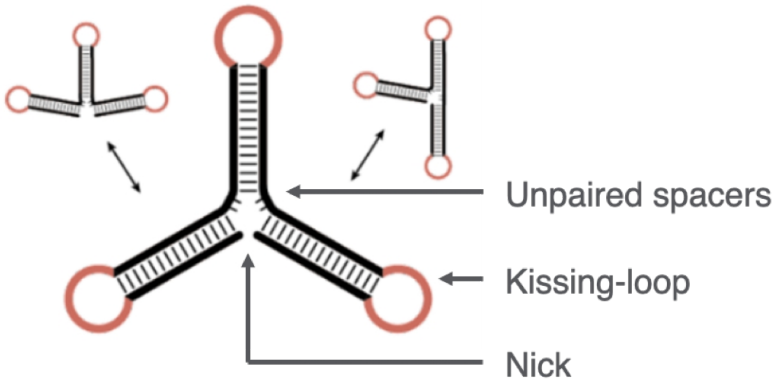


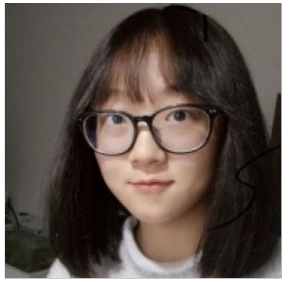
Chappell et al. 2015

- Well-understood, predictive folding models



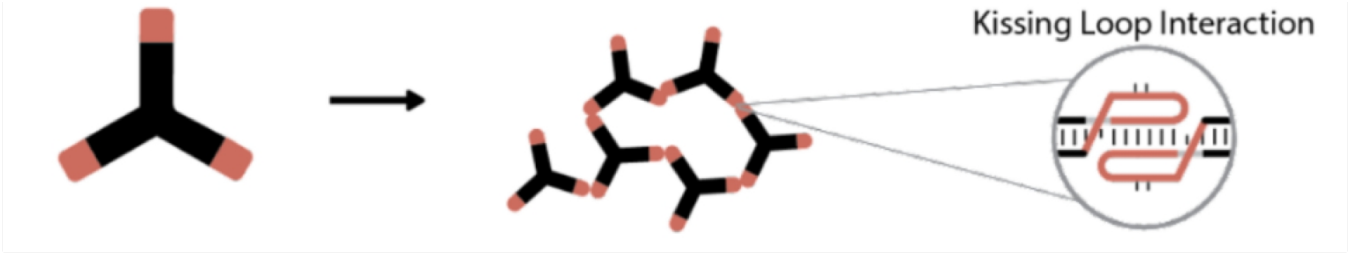
Phase separated RNA organelles

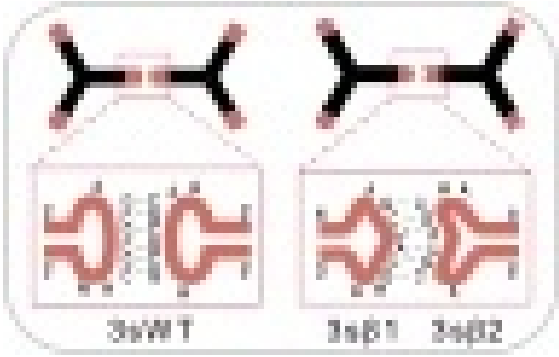




Shiyi Li

Anli Tang



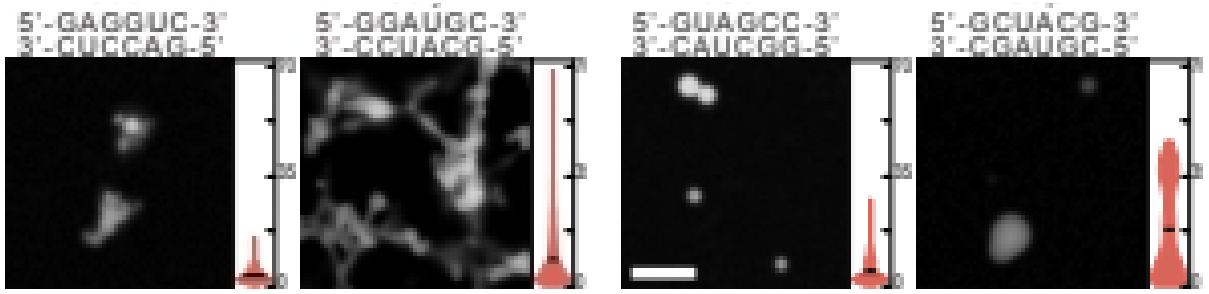


Stewart, Jaimie Marie, et al. "Modular RNA motifs for orthogonal phase separated compartments." bioRxiv (2023): 2023-10.

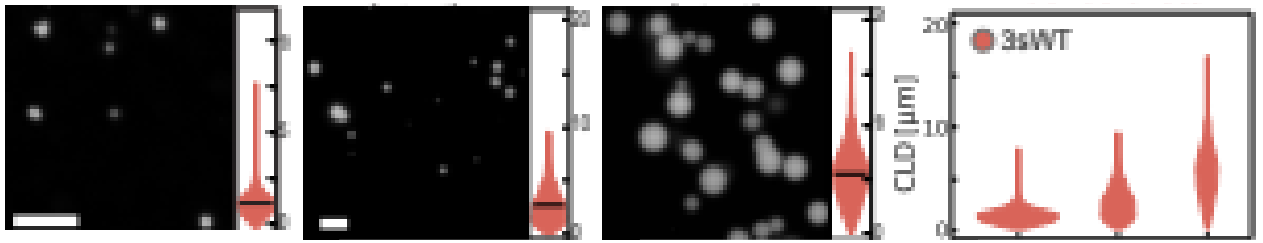
Fabrini, Giacomo, et al. "Co-transcriptional production of programmable RNA condensates and synthetic organelles." *bioRxiv* (2023): 2023-10.

Designing phase separated droplets through RNA nanostar motif optimization

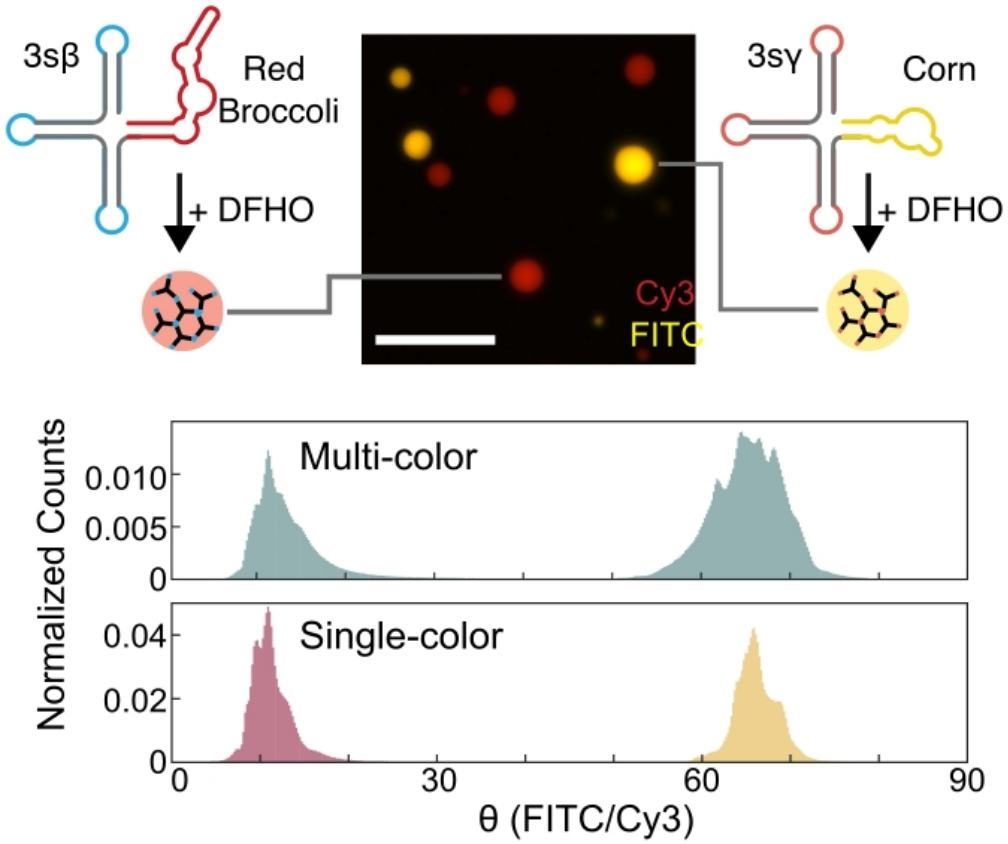
Different kissing loops influence morphology and yield



Different stem length influences condensate size

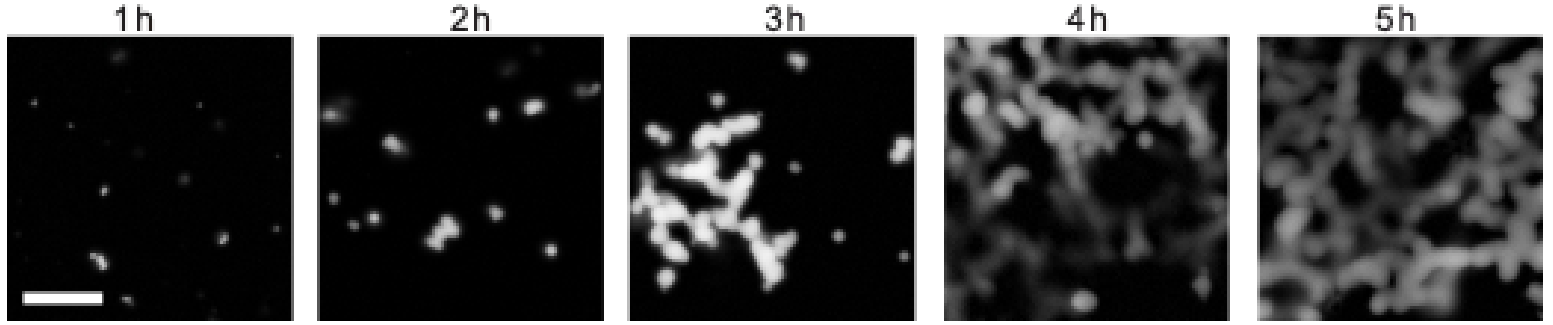


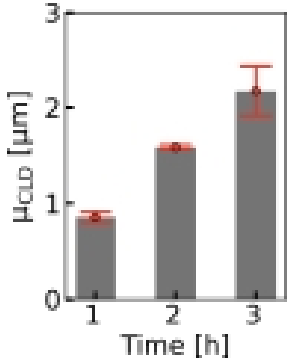
Orthogonal condensates for the recruitment of small molecules



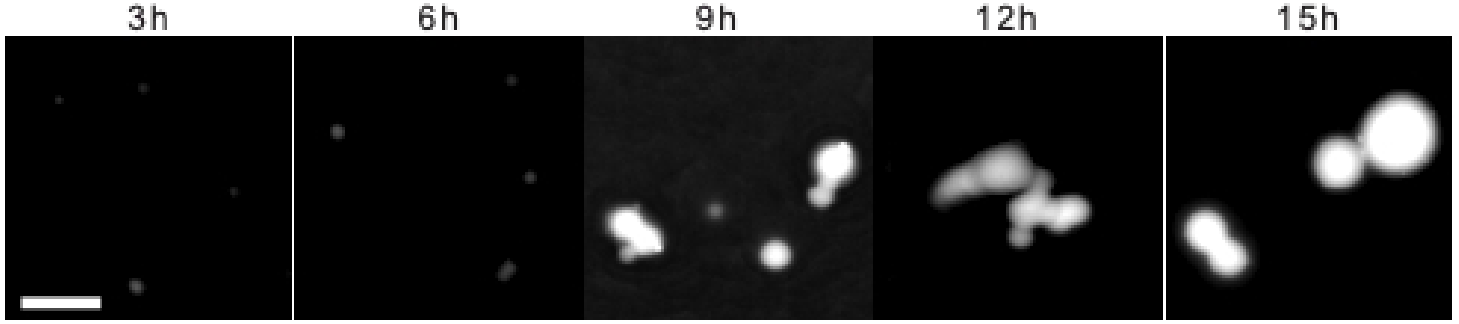
Isothermal formation of RNA droplets

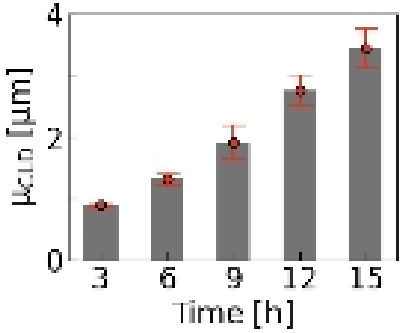
Transcription buffer



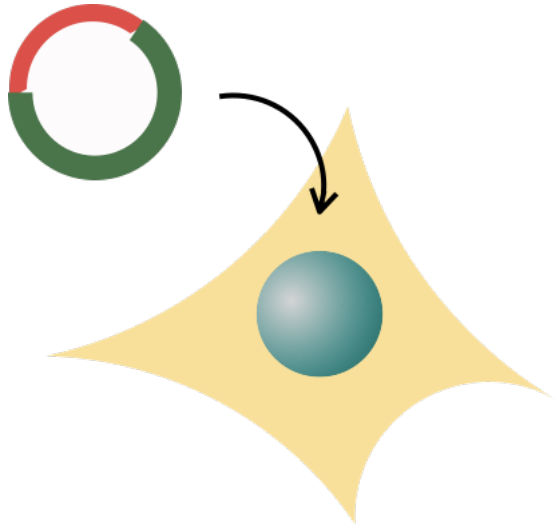


PurExpress

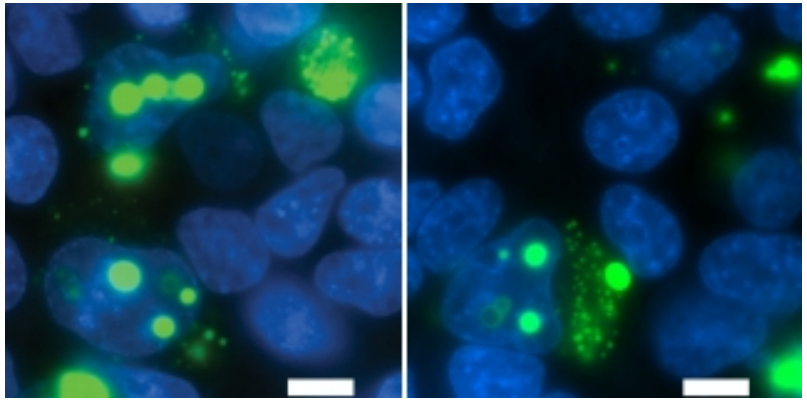




Looking forward: RNA organelles in living cells

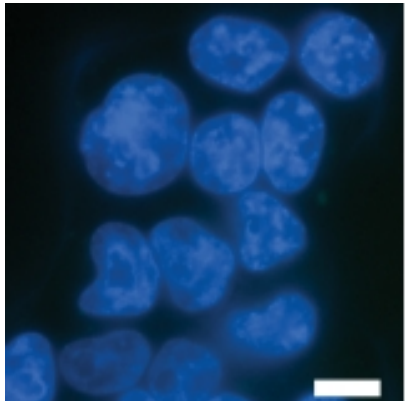


Preliminary data

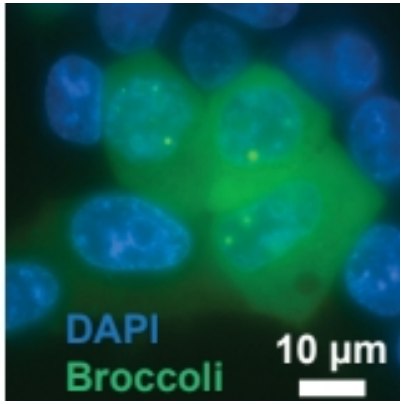


Hek293

Neg control



Fluorogenic aptamer



Future work:

- Localize organelles
- Recruit pathways
- Sense events

NSF Awards:
1938194 CAREER: Programming Dynamic Growth and Reconfiguration in Nucleic Acid Nanomaterials
2134772 FMRG:Bio : DNA & RNA Condensate Droplets for Programmable Separation and Manufacture of Biomolecules