Temporal Control Over Coacervation Using Ammonium Carbonate

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ABSTRACT

Biomolecular condensates and coacervates are liquid droplets formed by liquid-liquid phase separation. Condensates are involved in many cellular processes, but their mechanism of action is not well understood. Coacervates are often used as mimics of condensates, but there is a fundamental difference between them. In cells, biomolecular condensates appear and disappear, and their properties change over time in a process called **aging**. On the other hand, coacervates are normally studied as equilibrium structures whose properties remain constant.[1]

Here, we aim to develop a protocol for **aging in coacervates**. Our objective is to find a **general method** that works for all coacervates, so instead of targeting the chemical structure of the polyelectrolytes we target the salt –we used ammonium carbonate, a salt known to evaporate.

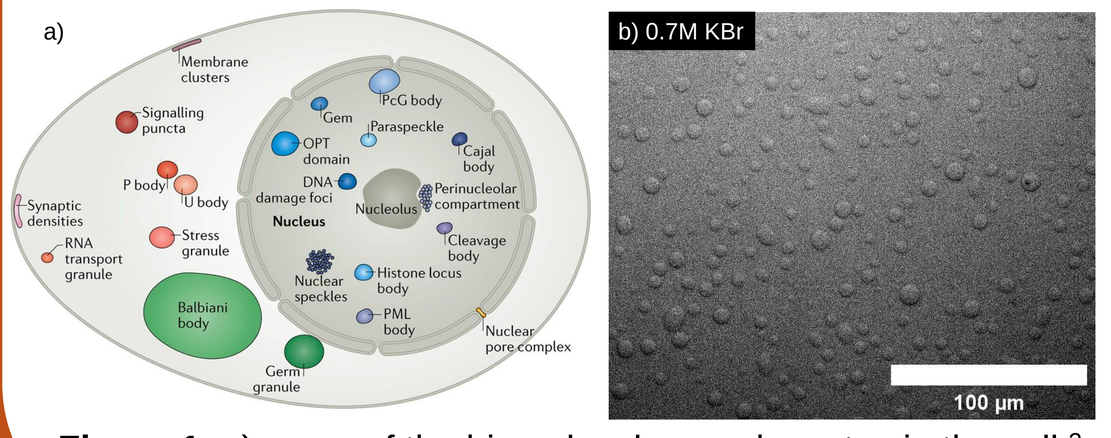


Figure 1. a) some of the biomolecular condensates in the cell.[2] b) complex coacervates

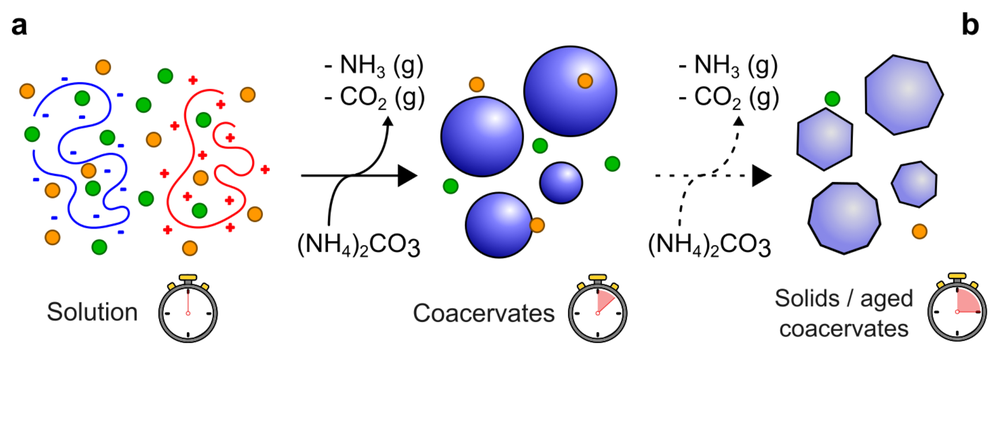


Figure 2. Strategy for temporal control used in this project

Effect of salt in coacervate formation

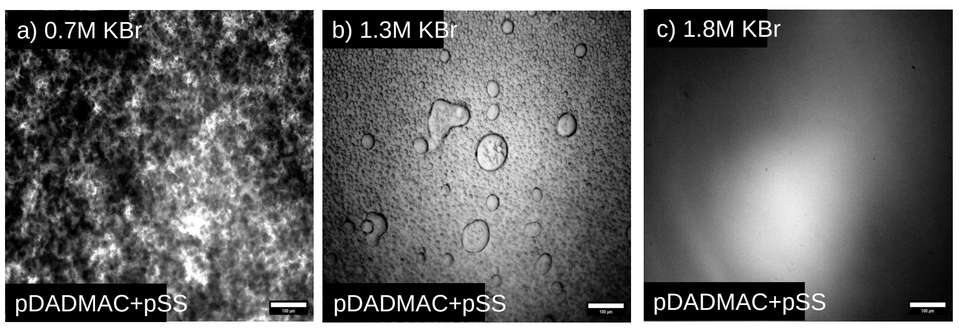


Figure 3. The formation of coacervates can be influenced by ionic strength.

Systems change from (a) solid to (b) coacervates to (c) solid as a function of salt concentration. When the salt concentration is higher than the **Salt resistances (SRs)** , coacervates disappear

Factors affecting the volatilization of $(NH_4)_2CO_3$

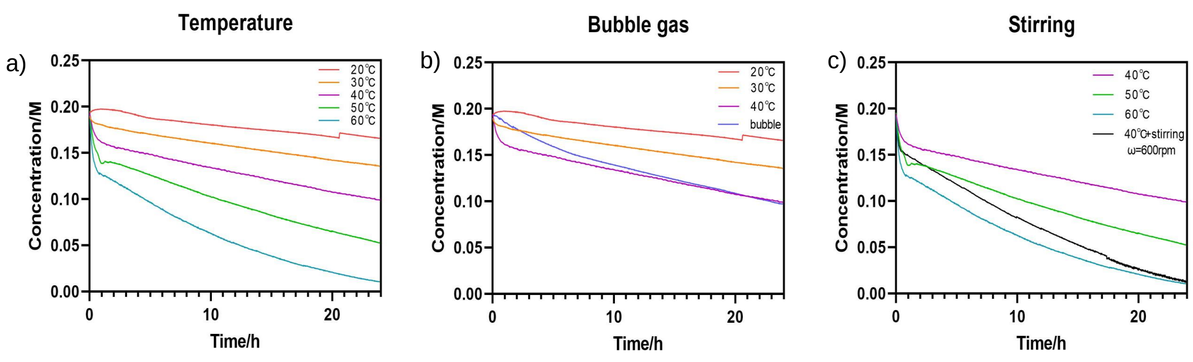


Figure 4. How temperature (a), gas bubbling (b), and stirring (c) can accelerate volatilization

Screening of different systems

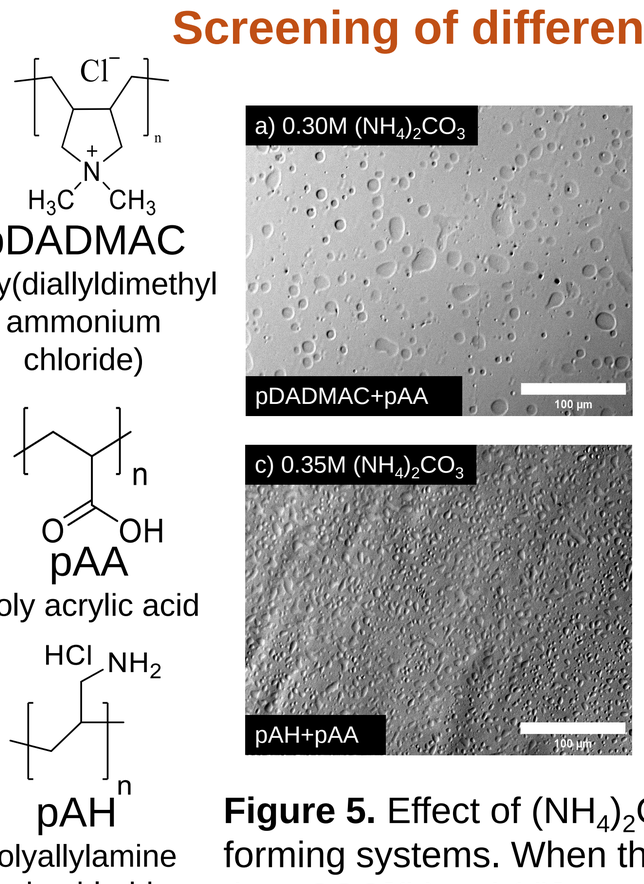


Figure 5. Effect of $(NH_4)_2CO_3$ in different coacervate-forming systems. When the salt concentration is higher than CSC[(b)and (d)], coacervates disappear.

pDADMAC+pAA+ $(NH_4)_2CO_3$

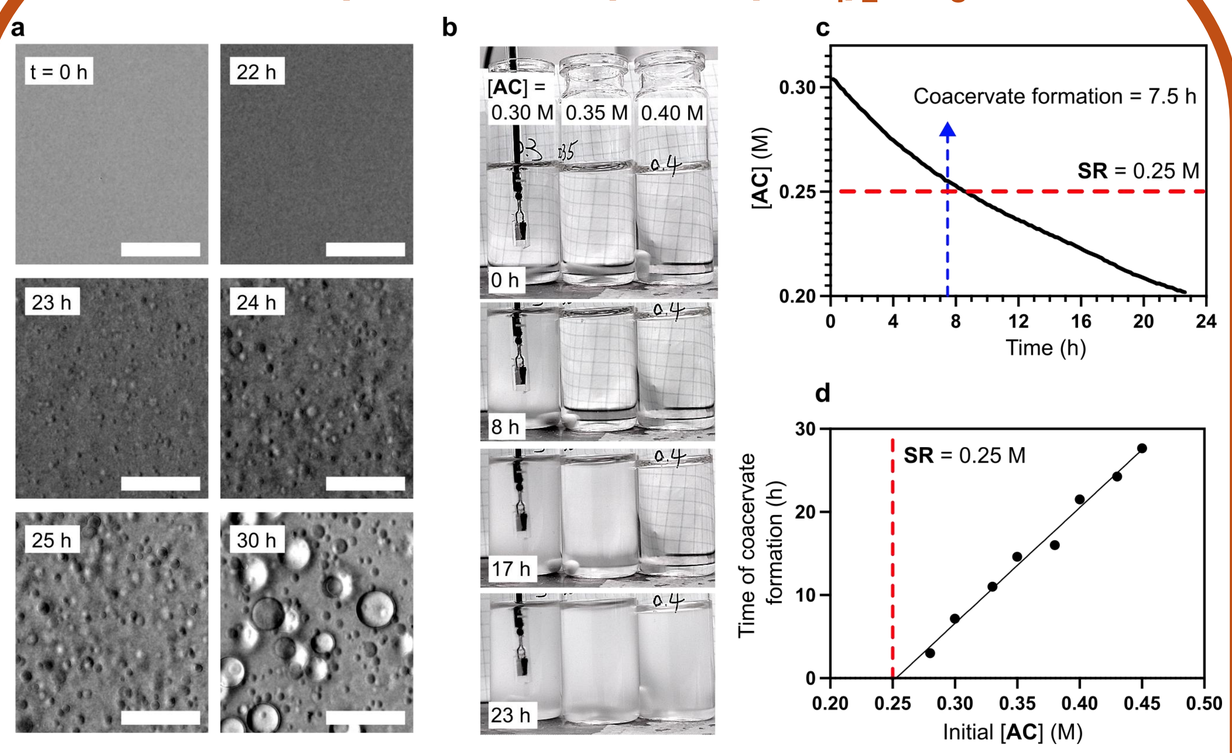


Figure 6. (a) microscope images at different stirring times, showing the appearance of coacervates. (b) The image suggests that due to the different initial concentrations of ammonium carbonate, higher concentrations require more time to reach salt resistance (SRs). (c) The concentration of ammonium carbonate after different stirring times can be determined from the measured conductivity data. (d) Based on the relationship between the appearance time of coacervates and different initial concentrations, we can estimate that the salt resistance (SRs) is approximately 0.25M.

Fluorescence Recovery After Photobleaching

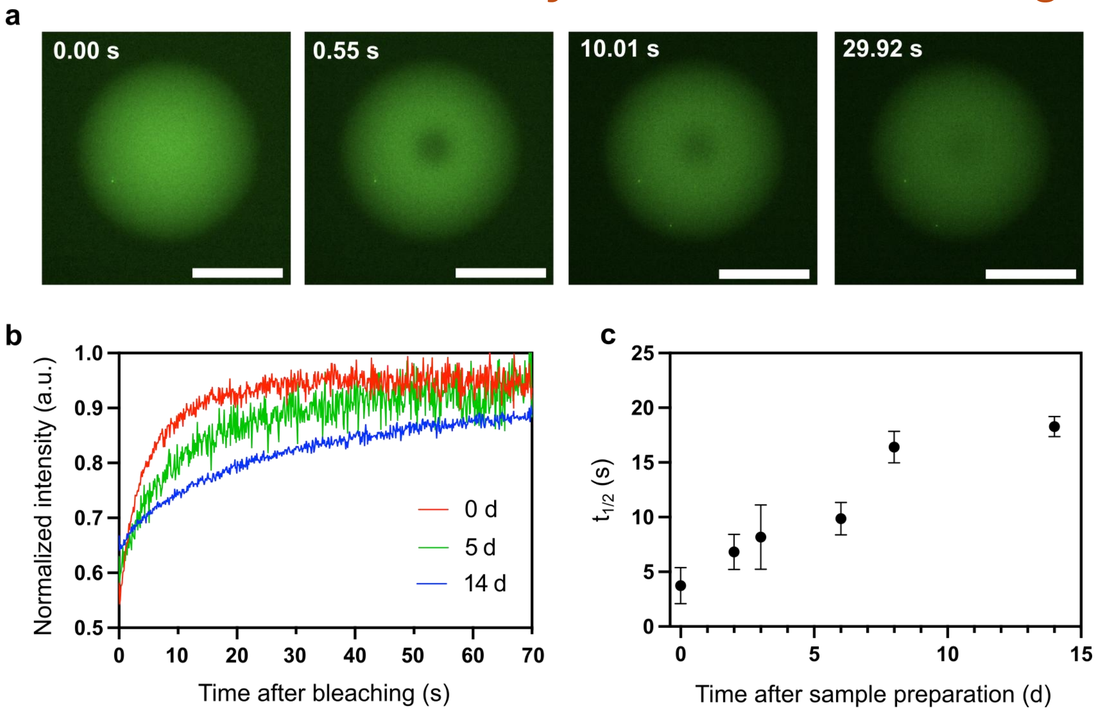
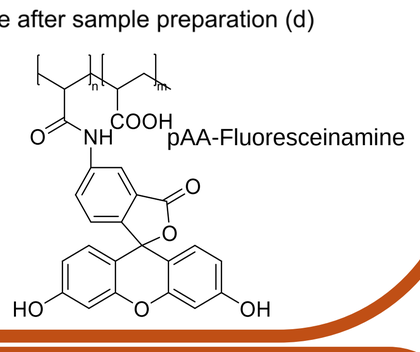


Figure 7. As the stirring time increases (and the ammonium carbonate concentration gradually decreases), the system becomes more solid-like.



References

1. Abbas, M. Lipiński, W. P. Wang, J. Spruijt, E., Peptide-based coacervates as biomimetic protocells, Chem. Soc. Rev. 2021, 50, 3690–3705.
2. Banani, S. F. Lee, H. O. Hyman, A. A.Rosen, M. K. Biomolecular Condensates: Organizers of Cellular Biochemistry. Nat Rev Mol Cell Biol 2017, 18, 285–298.
3. Lippincott-Schwartz, J.; Snapp, E. L.; Phair, R. D. The Development and Enhancement of FRAP as a Key Tool for Investigating Protein Dynamics. *Biophysical Journal* **2018**, *115* (7), 1146–1155.

Conclusions

1. Ammonium carbonate can modulate the properties of multiple coacervates
2. The evaporation rate of $(NH_4)_2CO_3$ can be controlled with different parameters
3. Evaporation can trigger coacervate formation and precipitation after controlled times
4. As the stirring time increases (and the ammonium carbonate concentration gradually decreases), the system becomes more solid-like.

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