# Liquid Crystal Phase Assembly in Peptide-DNA Coacervates as a Mechanism for Primitive Emergence of Structural Complexity

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## Abstract

Liquid-liquid phase separation (LLPS) has been proposed as a primitive compartmentalization mechanism at the origin of life. One major class of LLPS systems are complex coacervates, which have been assembled in the laboratory from potentially available biopolymers on early Earth such as peptides and nucleic acids. Such primitive coacervates have shown prebiotically relevant functions such as compartmentalization, scaffolding, and catalysis. However, how primitive coacervates could increase their structural complexity has not yet been well studied. Here, as a demonstration of a plausible mechanism by which primitive coacervate droplets can increase their structural complexity, we assembled and characterized a peptide-DNA coacervate droplet consisting of multiple co-assembled phases, in this case, a liquid crystal (LC) phase. LC phases in particular have been shown to promote chemical scaffolding and reaction selectivity, and we observed that the assembly of a LC-coacervate could be governed by prebiotically available environmental processes such as dehydration/rehydration or heat cycles. Structural complexity acquisition from LC phases could enhance the ability of primitive compartments to undergo selective molecular evolution.

## Coacervates as Primitive Compartments

Liquid-liquid phase separation (LLPS) is a ubiquitous process in modern cells that contribute to a variety of essential biological processes (Yoshizawa et al., 2020). Recently, LLPS such as polyester microdroplets (Chandru et al., 2020; Jia et al., 2021; Jia et al., 2019), aqueous two-phase systems (Mizuuchi & Ichihashi, 2020), or complex coacervates (Fares et al., 2020) have been proposed as primitive membraneless compartments at the origin of life. Complex coacervates in particular are produced from multivalent electrostatic binding of oppositely charged polymers, including those that may have been present on early Earth such as peptides or nucleic acids. While primitive coacervates can provide essential prebiotic functions such as compartmentalization (Frankel et al., 2016), and enhanced catalysis (Poudyal et al., 2019), there is still little known about how such compartments can increase their inherent structural complexity. Such structural complexity could have been essential to supporting enhanced molecular evolution of primitive membraneless compartments.

## Liquid Crystal Coacervate Co-Assembly

Liquid crystals (LC) are partially ordered structures and can imbue chemical reaction selectivity or rate acceleration to a system (Kansui et al., 1996); DNA LCs in particular have been shown to enhance non-enzymatic ligation of oligomeric nucleic acids into hundreds-of-bases-long polymers (Fraccia et al., 2015; Todisco et al., 2018). We sought to discover conditions in which LCs and coacervates could co-assemble as a potential mechanism to increase the compartments' structural and functional complexity.

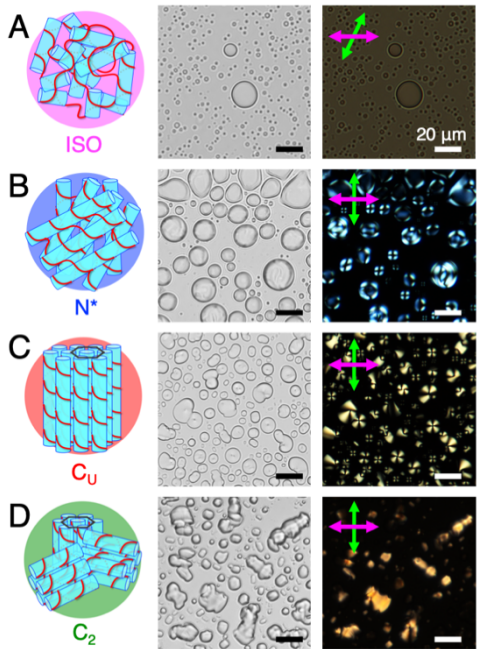


**Figure 1.** Structure sketches (left) and brightfield (middle) and polarized (right) optical micrographs of PLL/rDD (20 mM total charge) LC-coacervates with varied [NaCl]. (A) ISO (900 mM NaCl), (B) $N^*$ (800 mM NaCl), (C) $C_U$ (700 mM NaCl), (D) $C_2$ (600 mM NaCl). Colored arrows indicate polarizer positions; scale bars: 20 µm.

We observed that cholesteric ($N^*$) LC-coacervates composed of poly-L-lysine (PLL, a basic peptide) and rDD (a self-complementary DNA 12-mer forming blunt-end duplexes) could assemble in aqueous media (800 mM sodium chloride (NaCl)). LC phase formation is a sign that end-to-end linear aggregation of DNA duplexes occurs in concomitance with coacervation induced by PLL complexation. Decreasing [NaCl] resulted in transition of the cholesteric LC-coacervates to columnar (COL) LC-coacervates ($C_U$ and $C_2$). Increasing

[NaCl] resulted in assembly of liquid isotropic (ISO) droplets without LC (900 mM NaCl) (**Figure 1**) or disassembly to a droplet-less uniform phase (uniphase) (1 M NaCl). This is likely due to modulation of the electrostatic peptide-DNA binding interactions as a result of charge shielding at increasing ionic strength (Fraccia & Jia, 2020; Jia & Fraccia, 2020). The appearance of LC-coacervates resulted in more than two orders-of-magnitude increase in DNA concentration within the droplets, suggesting LC-coacervates could promote increased biopolymer uptake, potentially resulting in reaction acceleration for essential prebiotic processes and reactions.

## Environmental Control of LC-Coacervates

To discover how prebiotically relevant environmental factors, such as hydration or temperature, could affect LC-coacervate structure, we observed the LC-coacervate system under dehydrating/rehydrating conditions and at different temperatures, potentially caused by rainfall or diurnal/seasonal cycles. Dehydration resulted in transition from COL to N* LC-coacervate, through ISO droplets, and finally to the uniphase (**Figure 2**), which can be explained by concomitant increases in salinity due to the dehydration process (Fares et al., 2020). Rehydration resulted in recapitulation of the states in reverse order. We also observed a similar process (N* to ISO to uniphase) upon heating of the system, which also performed the reverse transition upon cooling (**Figure 2**). In this case, changes in temperature directly modulate DNA-DNA stacking and DNA-PLL binding interactions, causing such phase transitions.

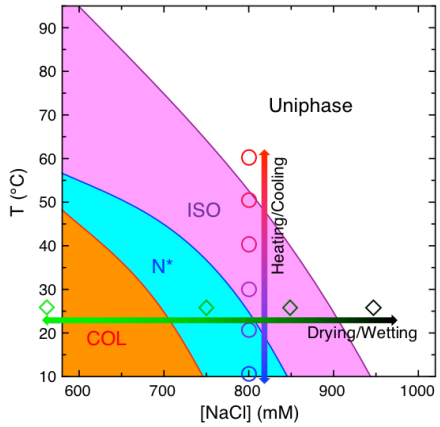


**Figure 2.** Phase diagram of the LC-coacervate system at different T and [NaCl]. Modulating T or [NaCl] transitions the system between mesophases such as from COL to N*, ISO, or uniphase (no droplets).

These cyclical structural changes show that LC-coacervate structure can be modulated directly by the environment; the LC-coacervates could potentially transition between functional and non-functional states (assuming that LC phases contribute some chemical selectivity or another function). As primitive chemical evolution required cyclical processes on early Earth, such environmental regulation could have promoted subsequent evolution of LC-coacervates, potentially resulting in further emergence of function.

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