LLPS in functional amyloids may unveil novel therapeutic targets for neurodegenerative diseases

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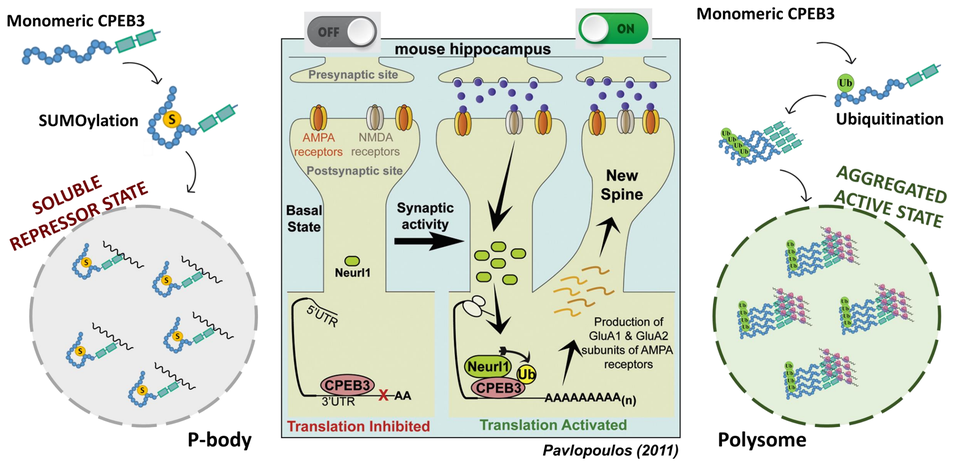
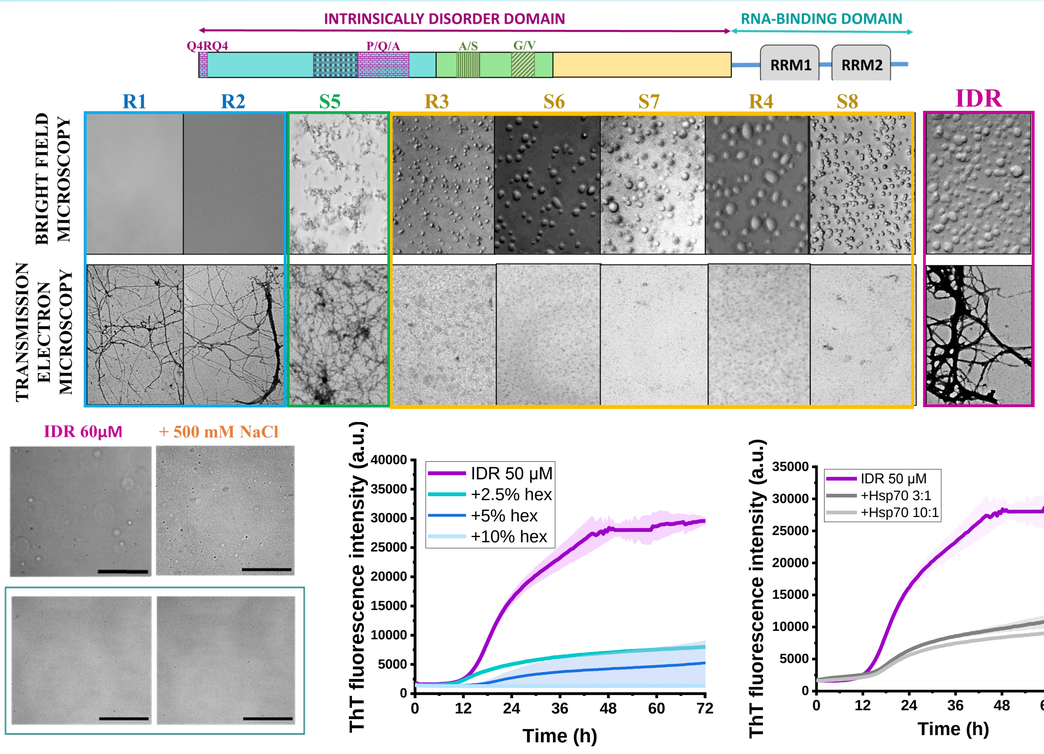
Liquid-liquid phase separation (LLPS) is driven by multivalent interactions that promotes the assembly of proteins into biocondensates[1]. An increasing number of **amyloid-forming proteins have been found to undergo LLPS**, emerging as a likely element in amyloid assembly regulation. In pathological amyloids, LLPS is an initial step of the toxic pathway as they mature into an irreversible aggregated state[2-3]. However, functional amyloids phase separate to control their physiological functions[4-5]. According to that, analyzing the **differences between functional and pathological LLPS** may open **new alternative therapies** and the discovery of new therapeutic targets[6]. Here, we have explored the sequence-driven molecular determinants behind the functional aggregation of human CPEB3 (hCPEB3), an RNA-binding protein key for memory persistence[7-8].

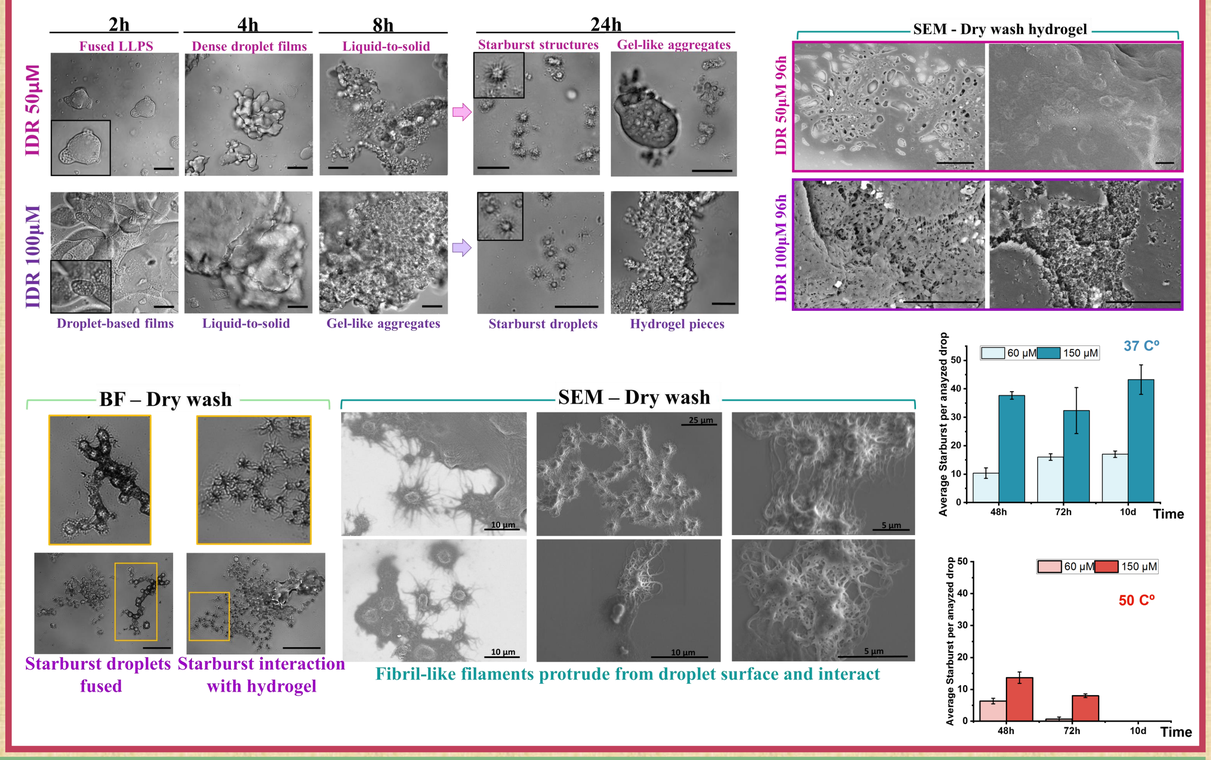
We found that the intrinsically disordered region (IDR) of hCPEB3 encodes both an amyloidogenic and a phase separation domain. LLPS of hCPEB3 relies on **hydrophobic interactions with ionic strength dependence**, and its droplet ageing process leads to a liquid-to-solid transition with the formation of a **non-fibril-based hydrogel**. Furthermore, we demonstrate the **differential behavior of the protein depending on its environment**. Under physiological-like conditions, it can establish additional electrostatic interactions with dissolved ions, increases the stability of its liquid droplets and follows a **condensation-based amyloid pathway.**



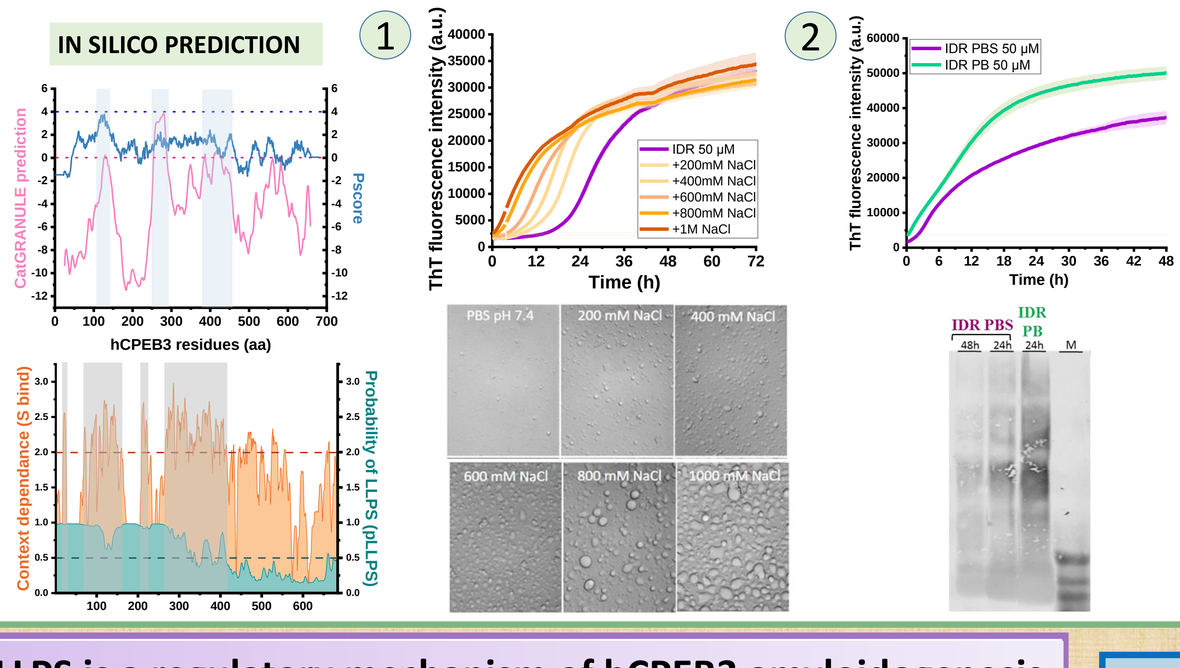
hCPEB3 has an amyloid-forming and a phase separation domain



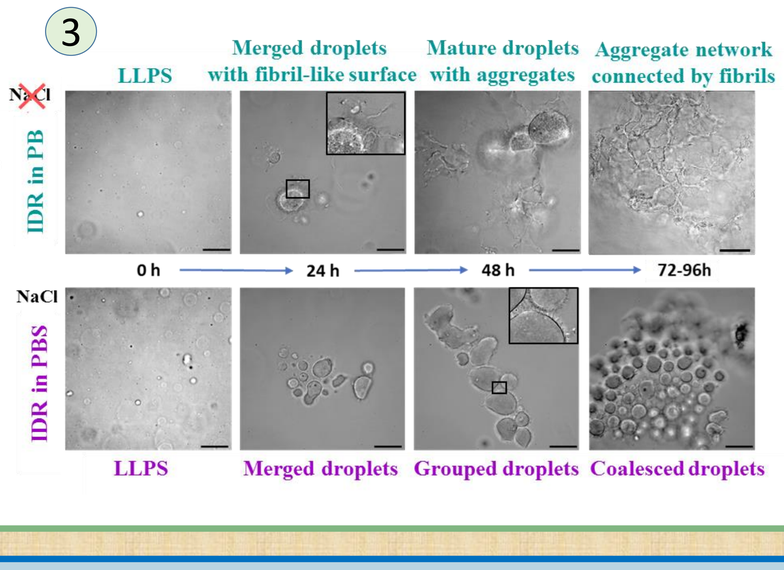
Droplet ageing forms hydrogel and starburst droplets

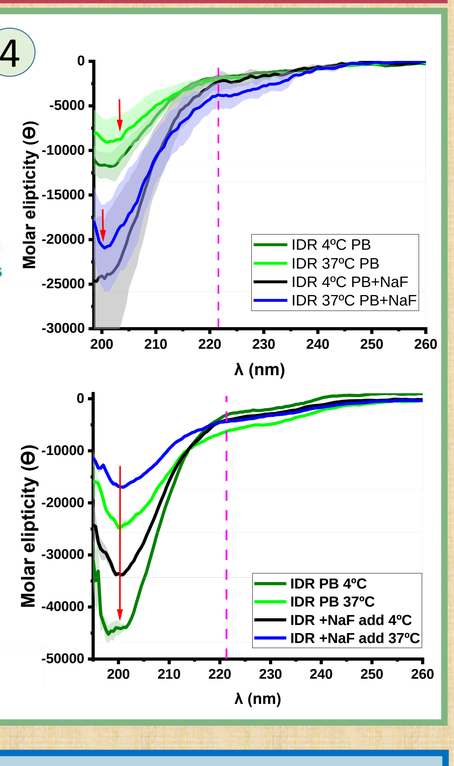


Context dependence modulates amyloid and LLPS formation in hCPEB3

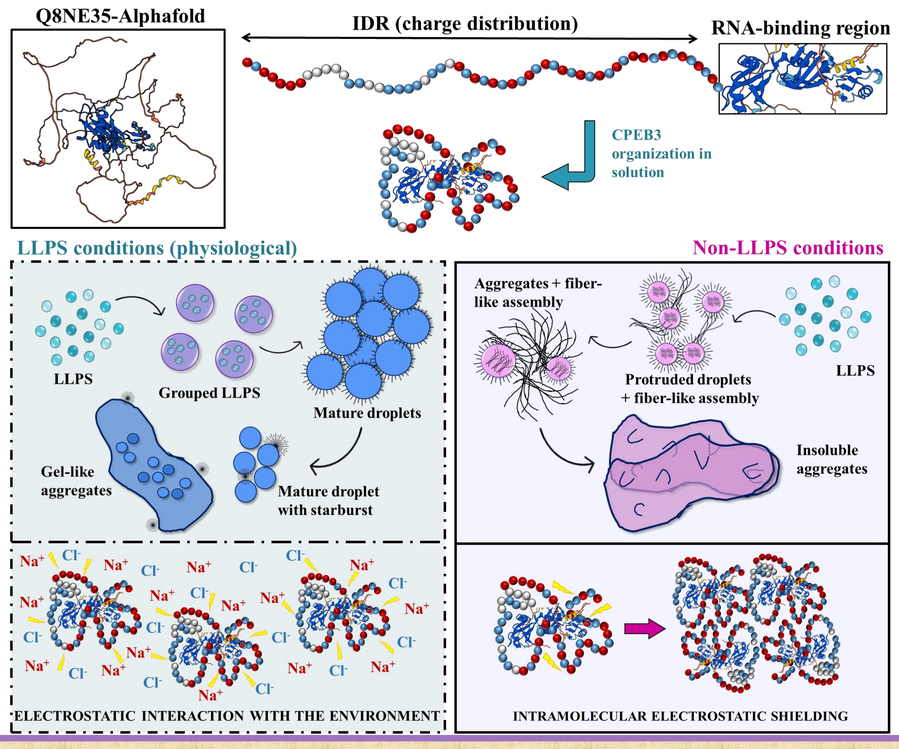


hCPEB3 functionality relies on the spatial and temporal **confinement of its amyloid formation** within its droplets, which are stable and long-lasting in **physiological-like conditions**.





LLPS is a regulatory mechanism of hCPEB3 amyloidogenesis



hCPEB3 regulation mechanism provides a therapeutic window to develop anti-amyloidogenic compounds based on droplet stability and LLPS modulation

References

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