Catalytic peptide-based coacervates for enhanced function through structural organization and substrate specificity

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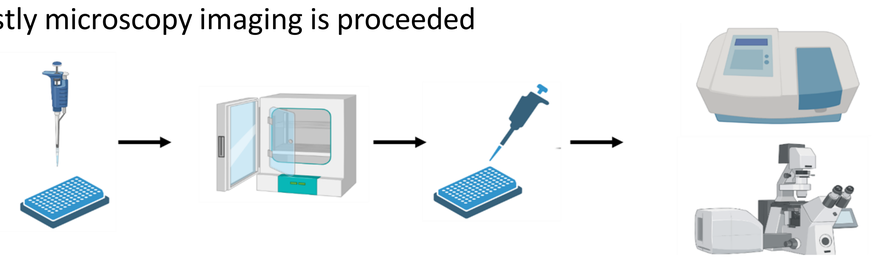
Bioinspired Peptide Systems Laboratory

Introduction

Cells use membrane-less organelles formed by liquid–liquid phase separation (LLPS) to spatially regulate biochemical reactions[1]. In polymer and colloid science, this process is known as coacervation and provides a framework for understanding biomolecular condensates. Nature exploits LLPS-driven coacervation in diverse contexts, such as β-carboxysome biogenesis[2]. Although coacervation has been widely explored to enhance enzyme activity, its application to catalytic peptides remains limited. Here, we show that LLPS-driven coacervation modulates P7, a 12-residue β-hairpin peptide with modest phosphatase activity and affinity for phosphorylated assemblies[3]. P7 coacervates adopt a fully folded β-hairpin conformation, forming densely packed internal domains, and compartmentalize guest molecules depending on charge and hydrophobicity, showing high affinity towards phosphorylated assemblies[4]. Remarkably, P7 coacervates yield a 15,000-fold increase in catalytic efficiency toward pNPP hydrolysis, underscoring their potential for advancing enzymatic applications.

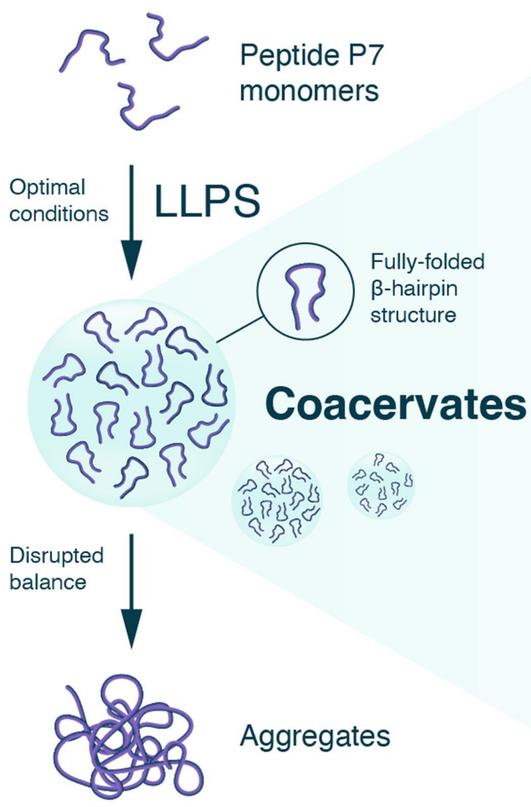
Methodology

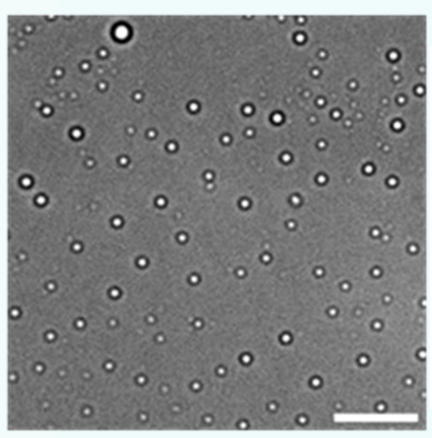
- Coacervation was achieved by mixing 5 mg/mL of P7 peptide[4] in 100mM of sodium phosphate buffer, containing 1M NaCl, at pH 8.0, to final peptide concentration of 1mg/mL
- This is followed by 1h of incubation at 27°C, leading to the formation of a milky-like state
- Lastly microscopy imaging is proceeded

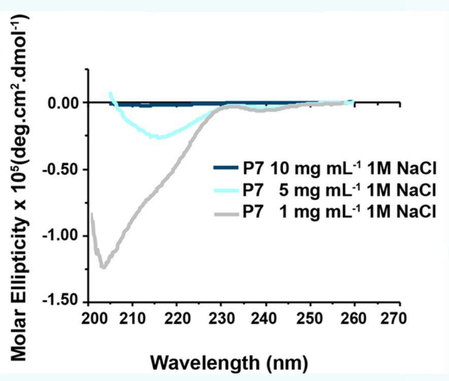


Results and Discussion

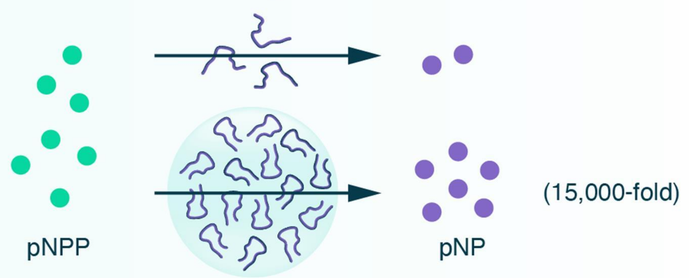
LLPS Propensity



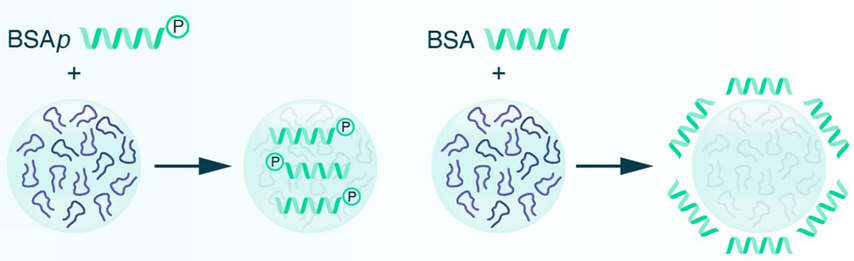




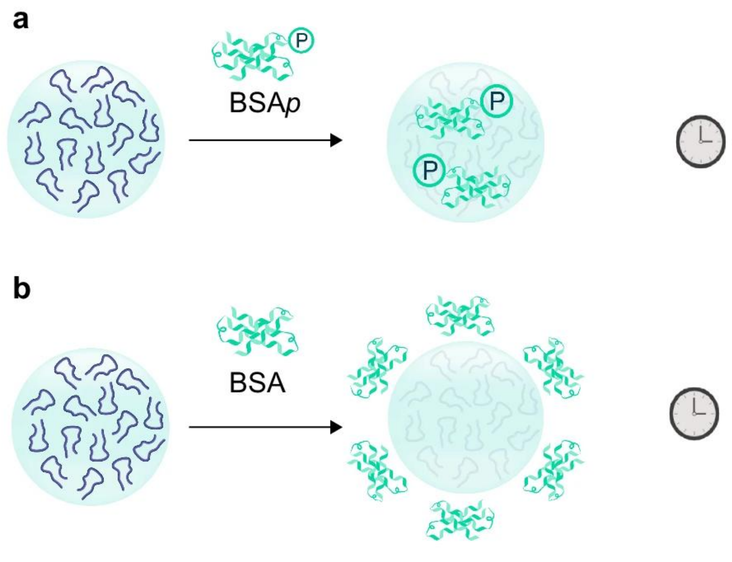
① Increased catalytic efficiency

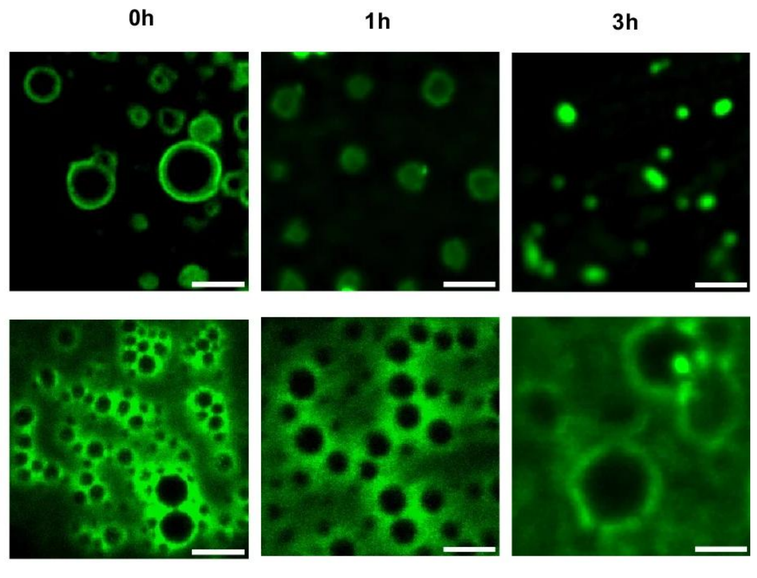


② Affinity-mediated molecular uptake



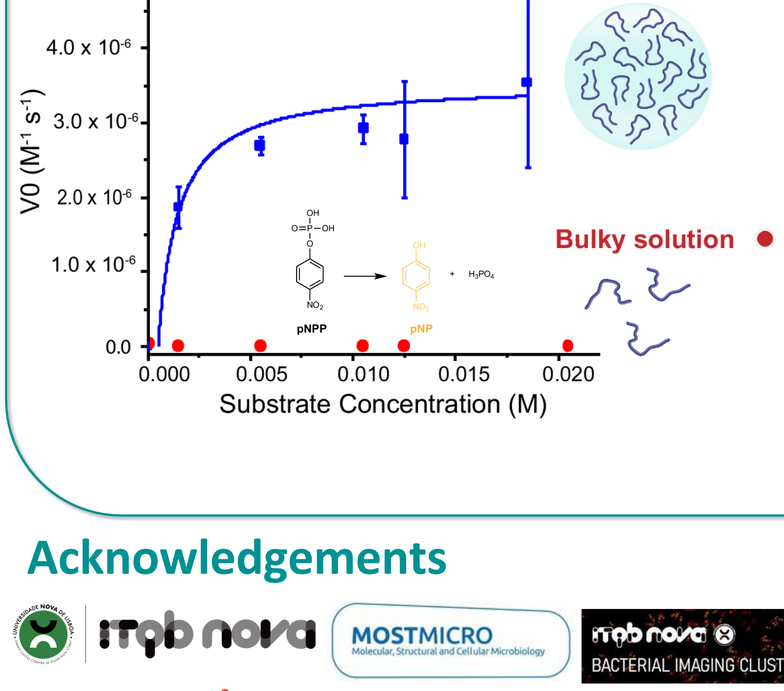
Affinity-mediated Sequestration





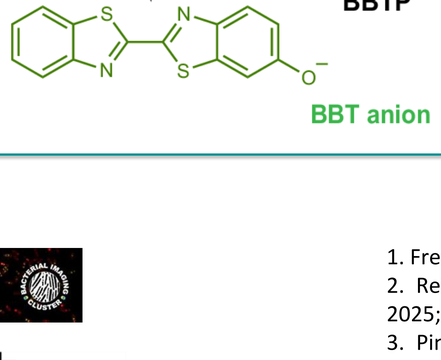
- Upon coacervate formation, P7's affinity for phosphorylated assemblies facilitates the sequestration of phosphorylated supramolecules (BSAp).
- P7 coacervates demonstrate a faster sequestration of phosphorylated BSAp compared to non-phosphorylated BSA.

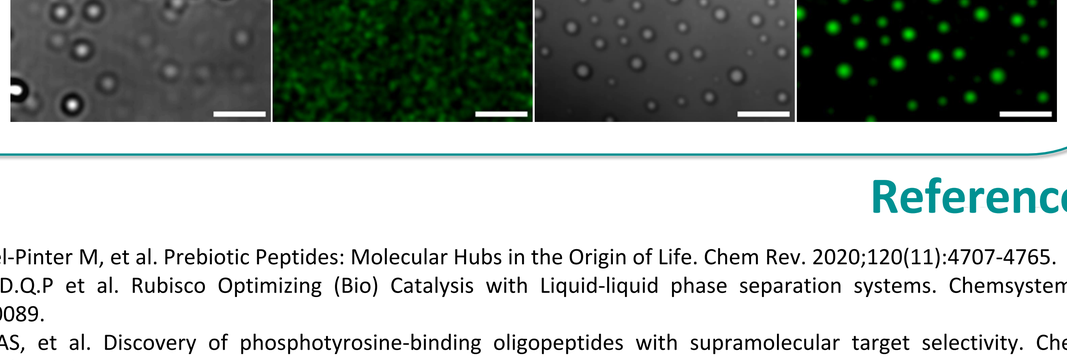
Catalytic Microreactors



Catalyst	k_{cat} (s^{-1})	K_M (M)	k_{cat}/K_M ($M^{-1}\ s^{-1}$)
P7-based Peptide Coacervates	(4.9 ± 0.6) x10^{-3}	(8.2 ± 3.2) x 10^{-4}	5.9 ± 0.2
P7 Peptide in solution	(1.0 ± 0.0) x10^{-5}	(1.4 ± 3.2) x 10^{-2}	(4.0 ± 0.3) x 10^{-4}

- 15,000-fold increase when P7 peptide coacervates are formed
- The catalytic reaction takes place within P7 coacervates





Acknowledgements

MOSTMICRO Molecular, Structural and Cellular Microbiology

BACTERIAL IMAGING CLUSTER

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