Peptide-based Coacervates as Evolutionary Roads of Catalytic Function

David Reis[1], Sara Pereira[1], Ana P. Ramos[1], Pedro M. Pereira[1], Leonor Morgado[2] and Ana S. Pina[1]

Drq.reis@itqb.unl.pt

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República,, 2780-157 Oeiras, Portugal
[2] Associate Laboratory i4HB – Institute for Health and Bioeconomy and UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER | ITQB.UNL.PT

https://doi.org/10.17952/37EPS.2024.P2191

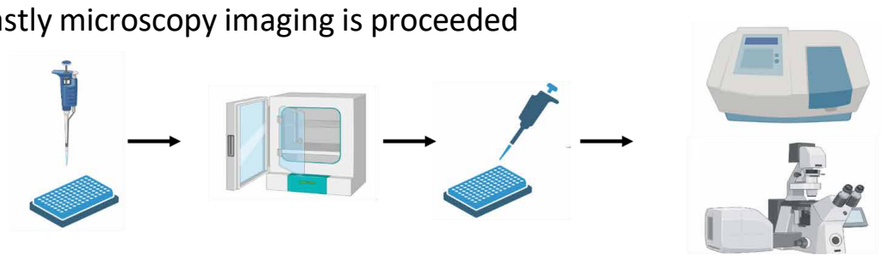
Bioinspired Peptide Systems Laboratory

Introduction

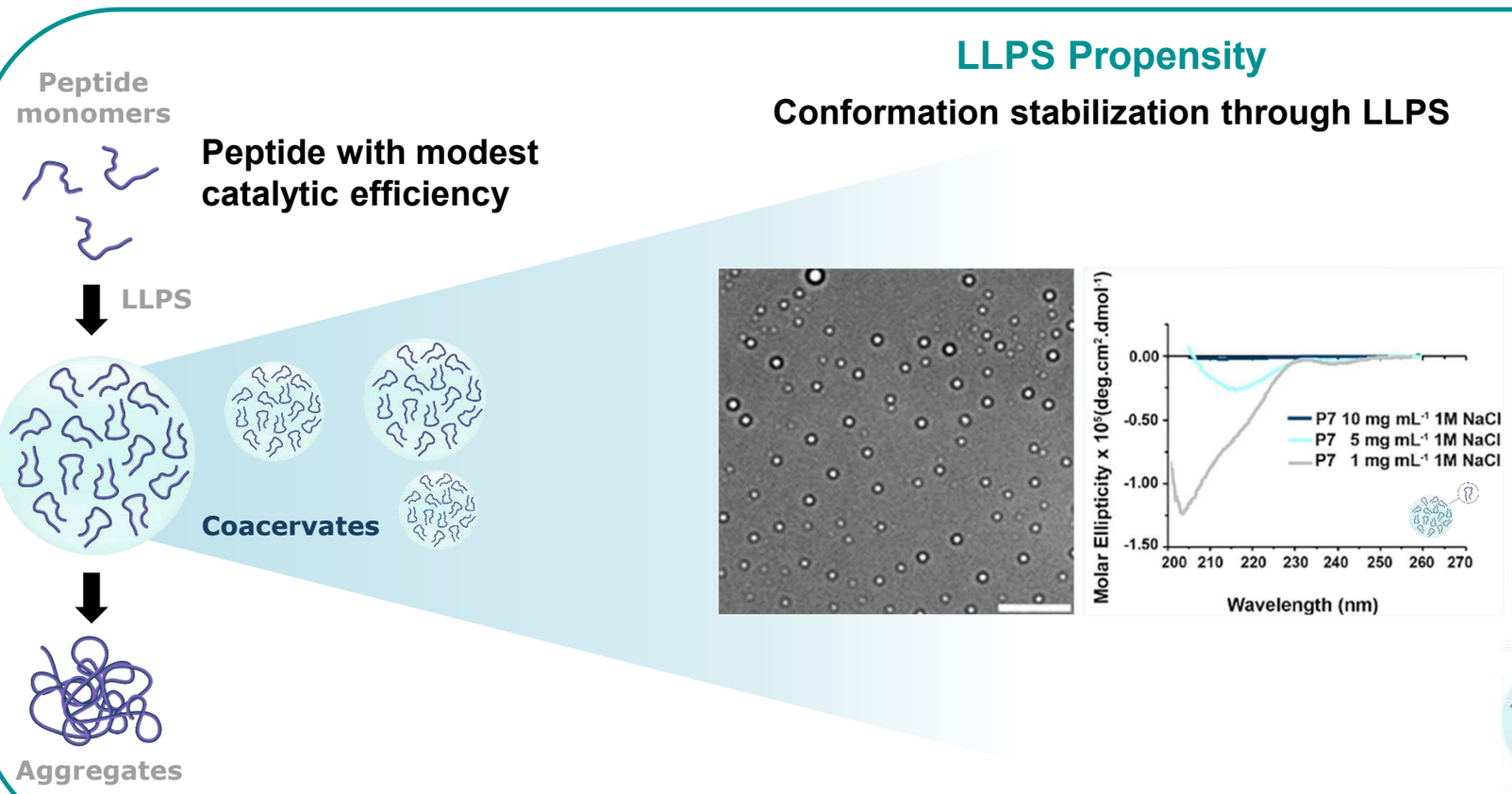
Scientists suggest that cooperative interactions among peptides facilitated the formation of enzymes, introducing functionality through sequence diversification[1]. While peptides serve as effective chiral catalysts in organic reactions, their utilization in aqueous reactions is still limited and remains a challenge in molecular engineering, arising from their conformational heterogeneity[2,3]. Coacervates, formed through liquid-liquid phase separation (LLPS), are considered primitive models of protocells, linked to the origin of life, shielding and concentrating oligomers[3]. Therefore, the compartmentalization of catalytic peptides appears to have great potential as a route to evolve catalytic function in small catalytically modest peptides, by constraining their conformational flexibility observed in aqueous solutions.

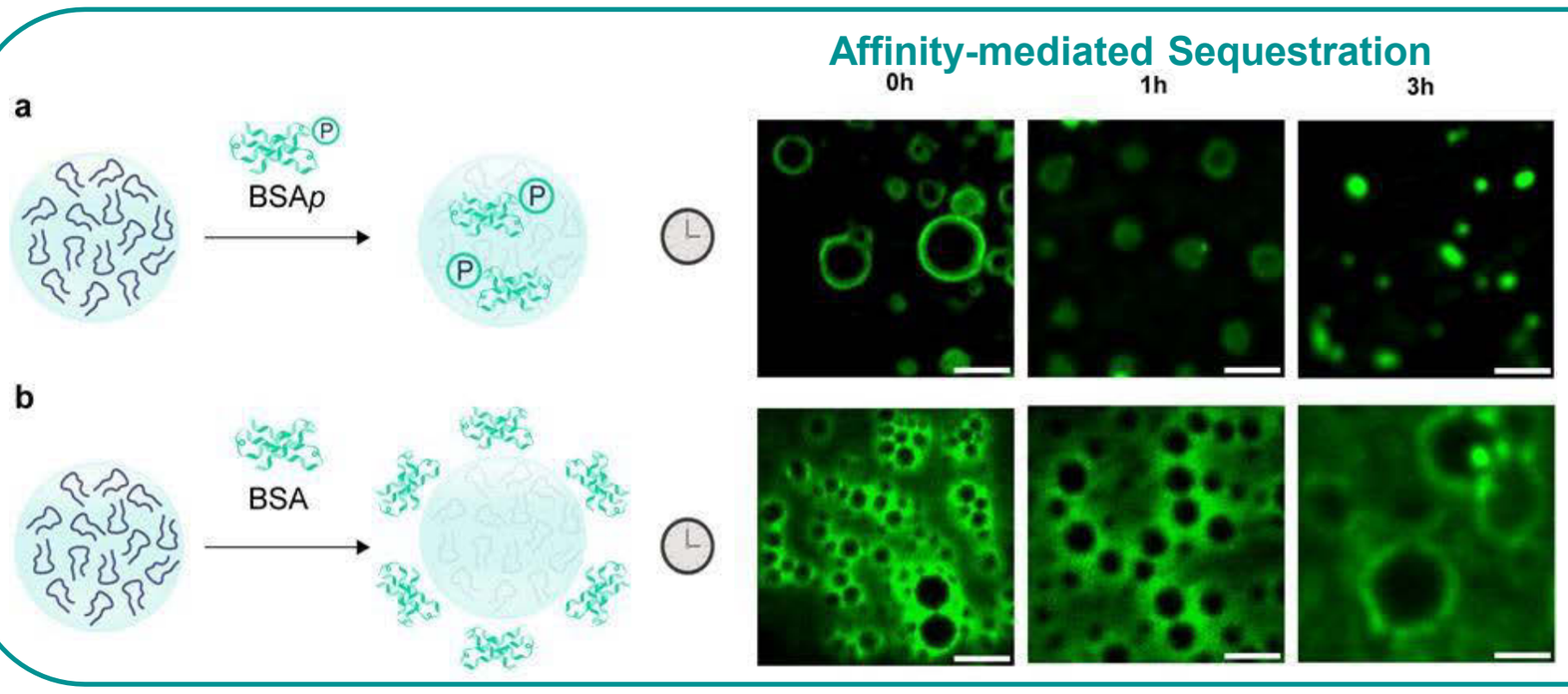
Methodology

- 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

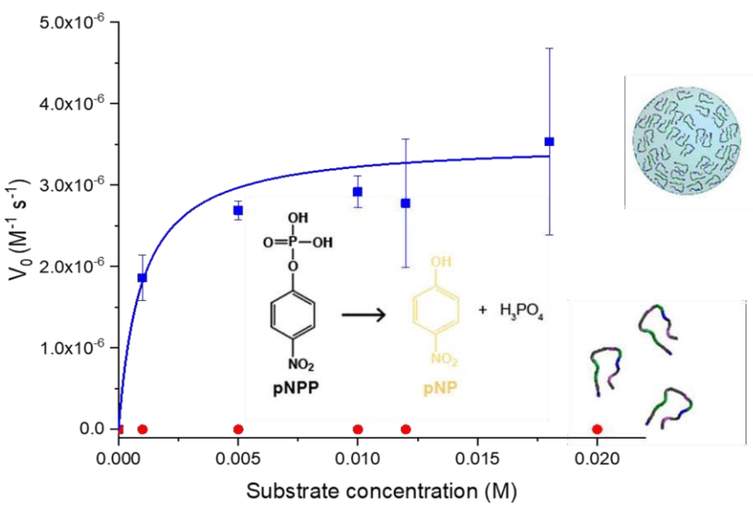


Results and Discussion



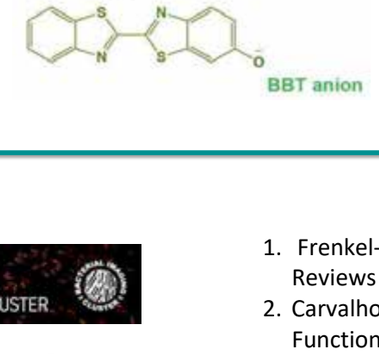


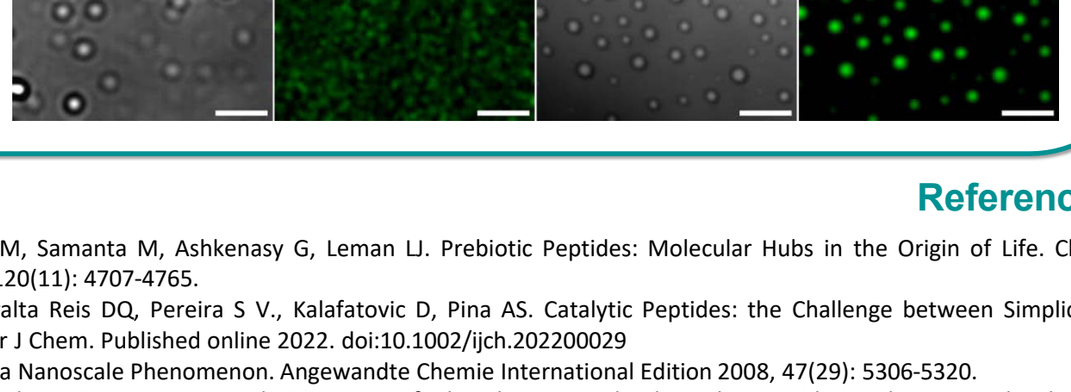
- Upon coacervate formation, P7's affinity for phosphorylated assemblies facilitates the sequestration of phosphorylated supramolecules (BSA*p*).
- P7 coacervates demonstrate a faster sequestration of phosphorylated BSA*p* compared to non-phosphorylated BSA.



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
ROYAL SOCIETY OF CHEMISTRY
UCIBIO Applied Molecular Biosciences Unit
NOVA SCHOOL OF SCIENCE & TECHNOLOGY
Institute for Health and Bioeconomy
Fundação para a Ciência e a Tecnologia

References

1. Frenkel-Pinter M, Samanta M, Ashkenasy G, Leman LJ. Prebiotic Peptides: Molecular Hubs in the Origin of Life. Chemical Reviews 2020, 120(11): 4707-4765.
2. Carvalho S, Peralta Reis DQ, Pereira S V., Kalafatovic D, Pina AS. Catalytic Peptides: the Challenge between Simplicity and Functionality. Isr J Chem. Published online 2022. doi:10.1002/ijch.202200029
3. Mann S. Life as a Nanoscale Phenomenon. Angewandte Chemie International Edition 2008, 47(29): 5306-5320.
4. Pina AS, Morgado L, Duncan KL, et al. Discovery of phosphotyrosine-binding oligopeptides with supramolecular target selectivity. Chem Sci. 2022;13(1):210-217. doi:10.1039/D1SC04420F