Design of Bioinspired Functional Materials Based on Liquid-Liquid Phase Separation for Biomedical Applications=

Thesis submitted for the degree "doctor of philosophy" by Tlalit Massarano
This work was carried out under the supervision of Prof. Ayala Lampel

Abstract

Living cells regulate chemical reactions through compartmentalization, creating specialized environments that provide spatial and temporal control. Inspired by this principle, this thesis develops bioinspired liquid–liquid phase separation (LLPS)-based microreactors that integrate enzymatic catalysis, compartmentalization, and peptide design.

As a model for enzymatic regulation, a polymer-based aqueous two-phase system, composed of PEG and Dex, was used to control melanin biosynthesis. Melanin is a biologically important pigment whose synthesis requires strict regulation, yet synthetic approaches often lack such control. Partitioning of tyrosinase into Dex-rich droplets provided spatial confinement, while a photocaged tyrosine derivative introduced temporal control. This enabled soluble, tunable melanin production with potential biomedical applications.

Biomolecular condensates offer dynamic environments for catalysis, yet the potential of minimal peptide systems to harness these features remains largely unexplored. To address this, short histidine-containing peptides were designed to form catalytic condensates. Two systems were established: Zn^{2+}-dependent condensates, where histidine-Zn^{2+} coordination activated water for ester hydrolysis, and Zn^{2+}-independent condensates, where histidine residues promoted catalysis via hydrogen bonding. These results demonstrate how peptide sequence and environmental context tune condensate reactivity.

Together, this work shows how LLPS-based systems can function as adaptive catalytic microreactors, providing spatial and temporal control over reactivity. Beyond advancing fundamental understanding, these systems suggest routes toward sustainable synthesis, controlled release, and biocompatible functional materials.