**Protein Immobilization using Complex Coacervates and Complex Coacervate Thin Films**

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## Abstract

Enzymes can enable a wide and growing range of chemistries in many industries, often outperforming synthetic catalysts. However, in order to be effectively deployed, enzymes must often be converted to heterogeneous catalysts. Protein immobilization enables this conversion by attaching the protein to a surface or encapsulating it within a material and can enhance the stability of enzymes. Complex coacervates have been demonstrated to be highly effective at encapsulating and stabilizing enzymes, and casting them into films enables immobilization onto surfaces. Therefore, this thesis demonstrates the use of complex coacervate thin films for the immobilization of enzymes and the synthesis of functional biomaterials and systematically probes methods to enhance the performance of these materials.

The first study presents a proof-of-concept demonstration of complex coacervate thin films for the synthesis of functional biomaterials. The immobilization method itself was all-aqueous, reducing the likelihood of enzyme denaturation, and facile, only requiring two steps: a single coating followed by crosslinking. A model biosensor was synthesized and demonstrated to have both high sensitivity and selectivity, and the immobilization method imparted increased thermal stability on the enzyme. From here, two directions were explored: the first looking at how protein properties affect their coacervation behavior and the second looking to optimize the performance of the complex coacervate thin films.

The second study proposes a method to quantify the surface charge distribution or the "patchiness" of proteins because, while patchiness has long been known to affect coacervation of proteins, it has until recently, not been quantified. A patchiness parameter that averaged pair correlations between neighboring points on the protein surface was shown to correlate with the coacervation behavior of proteins designed to have specific levels of patchiness with greater patchiness favoring the formation of complexes. Further work will enable this parameter to be incorporated with other protein properties in order to create robust predictive algorithms for protein-polymer coacervation.

The third and fourth studies aimed to enhance the performance and properties of complex coacervate thin films. The third study probed whether the morphology of these composite materials could be controlled and found that morphologies varied greatly as a function of the polyelectrolyte strength and the loading of the encapsulated molecule. The strongest interactions led to unfavorable precipitation, but weaker interactions led to micellization in both solution and the films. The fourth study aimed to understand how various polymer properties, including polyelectrolyte strength and monomer rigidity, affect the performance of complex coacervate thin films and whether this changes with protein charge. Both the catalytic performance and the thermal stability of the encapsulated lower charge protein varied significantly less with polymer chemistry than with the higher charge protein. This was driven by the fact that the lower net charge only allowed for weaker protein-polymer interactions, whereas the interaction strength for the protein with the higher net charge is driven by the polyelectrolyte strength of the encapsulant. Overall, strong interactions were found to favor greater catalytic activity but lower stability, while weaker interactions and greater monomer flexibility favored greater stability.

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