December 2020

Encapsulation and Stabilization of Biomacromolecules

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ENCAPSULATION AND STABILIZATION OF BIOMACROMOLECULES

A Dissertation Presented

by

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Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2020

Department of Chemical Engineering

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DEDICATION

To my wonderful husband for being the support to make this a reality and to my mom for being the inspiration to take on this project.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Sarah L. Perry, for her years of amazing support.

I want to thank Kathryn Rahlwes for her friendship and encouragement throughout this journey. You have been an amazing muse and helped me keep the delicate balance of being both a scientist and a person.

I wish to express my appreciation to all my friends and family who have supported my through this. A special thank you to those who have adventured with me: Matt Downing, Kelsey Keenan, Josh Baez Vigo, Seth Frisby, Ray Trammel, and Casey Lee-Trimble.

Finally, but not least, I would like to extend my gratitude to my dad for being a motivation to complete this degree and for being an example that it is possible to achieve such an accomplishment and make a difference. Watching you finish your Ph.D. spurred me to become the second generation of “Doctah Blochah.” I would not be the out of the box or creative thinker without you.

ABSTRACT

ENCAPSULATION AND STABILIZATION OF BIOMACROMOLECULES

SEPTEMBER 2020

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Recent work in the area of protein encapsulation has turned away from traditional methods of sequestration toward gentler, purely aqueous techniques. Among them, complex coacervation has become a topic of discussion. Complex coacervation is an all-aqueous liquid-liquid phase separation phenomenon dominated by electrostatic interactions and entropic gains. The use of coacervates as protein encapsulants has garnered much attention, but there has been little headway in determining a set of design rules. We considered coacervation between two oppositely-charged polypeptides and a biomacromolecule cargo to investigate the effects of changing aspects of the coacervating polymers and/or various solution parameters. We characterized the level of encapsulation and partitioning of three different model proteins as a function of ionic strength, pH, polymer chain length, and polymer charge density. Our results highlighted the importance of electrostatic interactions in driving protein uptake into the coacervate phase. While intuitive effects such as increasing protein charge facilitating uptake and increased salt concentration decreasing uptake due to electrostatic screening effects, we determined that the net charge and the distribution of charges on both the protein and the polymers dominated protein incorporation. For example, the presence of a cluster of cationic residues on the surface of lysozyme resulted in several orders of magnitude

higher protein incorporation than was observed for serum albumin and hemoglobin, which have a more isotropic distribution of charges. We confirmed this trend, comparing the encapsulation of two variants of caspase-6 with the variant with a cationic charge patch yielding a higher encapsulation efficiency than the other.

In addition to facilitating aqueous encapsulation of proteins, we hypothesize that complex coacervation can help to enhance the thermal stability of protein cargo through a combination of physical crowding and "soft" chemical interactions that mimic the naturally crowded environment of the cytosol. We tested this hypothesis using two model viruses, porcine parvovirus (PPV), a non-enveloped virus, and bovine viral diarrhea virus (BVDV), an envelope-virus. Accelerated aging studies at 60°C over the course of seven days demonstrated that coacervate encapsulation allowed PPV to retain more than three log higher levels of activity as compared to free virus in solution. For BVDV we did not observe significant stabilization, although we posit that this may be due to the presence of the envelope, which might already provide such protection. Overall, these preliminary results, obtained without considering the chemistry of the polymers, indicate the potential for using complex coacervation to enhance the shelf life of vaccines and biologics. This work sets the stage for future efforts geared towards understanding the specific ways in which the coacervate environment can affect protein and/or virus activity, including the potential for solvent removal.

These results for PPV indicate the potential uses of complex coacervates in applications such as drug delivery and therapeutics. However, the applicability of complex coacervates is not limited to the liquid phase. We explored the ability to electrospin solid fibers of a two-protein heteroprotein coacervate. These results give

useful insight as to how therapeutic protein-containing complex coacervates might be formulated and then processed for applications such as advanced wound dressings.

Beyond protein encapsulation, we explored the kinetics of binary complex coacervation utilizing a liquid handling robot. We were able to monitor the complexation of two peptides over time through turbidity measurements. These data described how factors such as system asymmetry and the addition of buffer or salt play critical roles in the complexation of two peptides. We also examined the phase behavior of more complex systems of two industrial polymers and a mixture of surfactants. Together, we garnered a broader understanding of the phase space of complexation with an emphasis on high throughput formulation.

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