1 Project Summary

1.1 Overview

The design and discovery of new surface-active polymers that self-assemble on solid substrates to form brush layers will have a major impact on numerous bioengineering and materials science applications. Nature can serve as a valuable tool in this research due to its unique capability of synthesizing complex biopolymers with a high level of control over composition, structure and function. For example, Nature utilizes 20 simple amino acids with varying individual physiochemical properties to create a remarkable array of complex polypeptides and proteins that perform numerous essential functions. Through recombinant DNA technology, there exists the potential to use the natural protein synthesis machinery of an organism to produce a synthetic polypeptide with an exactly specified amino acid sequence, thus controlling the polymer's composition and structure at a level unequaled by conventional organic polymer synthesis techniques.

Groundbreaking work from several research groups has demonstrated that recombinant DNA technology can be used to produce unique synthetic structural protein polymers for tissue engineering and material science applications. However, the current techniques are inefficient for the streamlined production of *poly(amino acid)* (or PAA) *brush-forming diblock copolymers*. The design and production of such polymers with optimal brush-forming properties requires an *iterative approach* in which the molecular weight of each block is varied to produce optimal surface coverage and brush extension. Thus, if advances are to be made in the discovery and design of novel PAA brush-forming polymers, the development of new cloning strategies is necessary in order to simplify the process of synthetic gene-assembly and subsequent PAA expression in genetically engineered organisms.

The long-term goal of this research is to synthesize new and unique brush-forming PAA's for biomedical and material science applications. *Therefore, the objective of this project is the development of a new cloning strategy customized for the production of unique poly(amino acids) that are surface-active and self-assemble on metal oxide surfaces to form brush layers*. Improving upon the limitations of previously published

methods, our proposed strategy will be more flexible and will enable a more rapid approach to achieving optimal brush-forming compositions. Our *rationale* is that once this streamlined cloning strategy is made available to the scientific community, it will reduce the barrier that currently exists between biotechnology and materials science, ultimately leading to a significant increase in the ease in which we and other investigators will be able to produce surface-active PAA's for a wide variety of fundamental studies and practical applications.

This is a highly multidisciplinary project that combines biotechnology, polymer science and surface adsorption science. Dr. Kevin Van Cott's research group is leading this effort with the development of these new cloning strategies and the biosynthetic production of the specifically designed PAA's. Dr. Richey M. Davis' research group is providing expertise in the area of polyelectrolyte solution properties and sorption measurements. Dr. William A. Ducker's research group is providing expertise in the area of polymer and surfactant adsorption, and will conduct the experiments to characterize PAA adsorption using atomic force microscopy and other spectroscopic techniques.

1.2 Objective and Specific Aims

The specific objective of this research is to develop new cloning strategies that can be easily adapted for the rapid production of modular, sequentially-modifiable, surface-active PAA's. The main advantage of our new cloning strategy is that *modules* of DNA that encode for short PAA blocks can be easily inserted directly into a commercially available and unmodified expression vector and the insertions can be made *repeatedly* until the gene encoding for a polymer of desired molecular weight and composition is attained. To accomplish our objective, the following specific aims are proposed:

1. ***Design a PAA sequence that will self-assemble on aluminum oxide surfaces to form brush layers, and produce this PAA in genetically engineered E. coli***
   The working hypothesis for this aim, based on prior work, is that a PAA sequence with an acidic anchor block and a proline-rich or zwitterionic tail block will self-assemble on aluminum oxide surfaces to form effective brush-like structures. To test this hypothesis, a PAA diblock, consisting of a glutamate-based anchor block and proline-based tail block, will be biosynthetically produced and isolated using conventional recombinant protein production methods. We expect to confirm the

feasibility of biosynthetically producing a unique PAA that has the potential to form sufficient brush layers on metal oxide surfaces.

2. ***Develop a new cloning strategy to meet the need for a simple and universal method for constructing the genes required for producing specifically designed, high molecular weight brush-forming PAA's in genetically engineered E. coli***
   A more streamlined cloning strategy will significantly increase the flexibility with which high molecular weight brush-forming PAA's of defined amino acid sequence and composition can be produced within genetically engineered *E. coli*. We will demonstrate a newly developed cloning strategy that is modular and allows for the modification of the PAA gene as it exists within the expression vector. We will use this strategy to construct the gene encoding for a PAA with a short acidic anchor block and a long zwitterionic tail block, and this PAA will be biosynthetically produced. We expect to confirm the feasibility of applying our cloning strategy to the biosynthetic production of a unique PAA that has the potential to form optimized brush layers on metal oxide surfaces.

The research presented is innovative in its synergistic combination of biotechnology, polymer science and surface science to produce and evaluate novel polymers. With the conclusion of this project, we will have demonstrated that the newly developed cloning strategies can be easily adapted for the production of a wide variety of PAA's for self-assembly. The simplicity of the strategy means that research groups will no longer need to develop their own customized molecular biology reagents. Therefore we, in addition to other groups, will be able to rapidly produce these PAA's and contribute to both fundamental and applied studies in PAA self-assembly phenomena.

1.3 Significance of Research

The published literature on brush-forming polymers being used in a variety of applications is rapidly growing. These applications include biomolecule separations,[1,2,3,4] diagnostic and sensor devices,[5,6,7,8] biomaterials and tissue engineering,[9,10,11] specialty coatings and adhesives,[12,13,14] microelectronics processing and nanotechnology.[15,16] While synthetic organic polymers have a well-defined utility, the use of biotechnology to produce brush-forming PAA's will exponentially increase the number of available applications. For example, a future application for this technology may include the production of polymers for specialty coatings that incorporate catalytic domains of enzymes or domains that stimulate (or inhibit) biomolecule adsorption. In spite of these potential applications, there still exists a critical gap that centers on the inexpensive and

efficient production of brush-forming PAA's. Solid phase peptide synthesis cannot be used because of the molecular weight limitations inherent in that process. Additionally, recombinant DNA assembly strategies that have been developed by Cappello *et al.*,[17] McMillan *et al.*,[18] Meyer and Chilkoti,[19] and Won and Barron[20] for synthetic structural protein polymers are not suitable for the iterative process of producing homologues where the molecular weight of each block needs to be easily varied to determine conditions for optimal brush formation. *Therefore, the presented research is significant because we will demonstrate a new cloning strategy that is specifically designed for the facile production of optimized brush-forming PAA's.* Concurrently, because the polymer composition and molecular weight can be *exactly* controlled and the new strategy allows the molecular weight of each block to be sequentially modified, this work will pave the way for breakthroughs in fundamental material science research studying self-assembly phenomena. Hence, the work presented here will have both practical and intellectual impacts. The flexibility and ease with which our technique can be adapted makes it an extremely attractive investigative tool that can be applied to many applications.

1.4 Outline of Chapters

This dissertation is divided into several chapters. Chapter 2 briefly discusses the main principles pertinent to this work as well as a review of relevant literature. Presentation of experimental work begins in Chapter 3. In this chapter, the design and production of a unique brush-forming PAA using conventional recombinant DNA techniques is presented. A manuscript accepted for publication in *Langmuir* is presented in Chapter 4. This work investigates the ability of a PAA related to the construct produced in Chapter 3 to form brush layers on alumina surfaces. Due to the limitations of the cloning methods used in Chapter 3, a new cloning strategy customized for the production of brush-forming PAA's was developed and is introduced in Chapter 5. In Chapter 6, this strategy is used during the production of a PAA designed to form optimal brush layers. A manuscript submitted to *Biomacromolecules* based on the work presented in Chapters 5 and 6 is located in Appendix A. Chapter 7 discusses the main conclusions drawn from the completed work, as well as recommendations for future experiments and possible additional applications. Though somewhat unrelated to the main focus of this

dissertation, Chapter 8 discusses a separate project that was a collaboration effort with the U.S Department of Defense and Luna Innovations. The aim of this project was to demonstrate the feasibility of using phage display technology to develop a liposome-based immunoassay for the rapid detection of biological toxins.

1.5 References

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