

ENGINEERING PROTEIN-BASED MATERIALS  
THROUGH COILED-COIL MOTIFS

Thesis by  
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In Partial Fulfillment of the Requirements  
for the Degree of  
Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California  
2007

(Defended January 23, 2007)

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## ACKNOWLEDGEMENTS

First, I thank David Tirrell, for being a great mentor and advisor, and for his constant guidance and support. I still remember the exciting moment five years ago when he welcomed me to be part of his research group. Dave's patience helped me safely struggle through those early rough years. His insight and open-mindedness are a great inspiration for me to pursue science with passion. I have been given a lot of opportunities to work on various projects and interact with people from diverse scientific backgrounds. The interdisciplinary nature of research in Dave's group is one of the things that have benefited me most these past several years. In addition, I am grateful to Dave for advising me on my career choices and painstakingly teaching me how to write papers.

I thank my thesis committee, Dennis Dougherty, Linda Hsieh-Wilson and Anand Asthagiri, for their valuable advice and comments on my thesis. I appreciate the way my proposals were challenged by these scientists during the candidacy and proposal examinations. I would also like to thank Steve Quake, Scott Fraser and Alireza Ghaffari for their generosity to allow me to use their facilities.

I also thank the former and current group members. When I first joined the group, Kent Kirshenbaum taught me the basic molecular biology techniques. Julie Liu shared her experience on culturing of mammalian cells with me. In particular, I feel the most profound gratitude towards Michael Diehl. As we worked with each other, joked with each other, and occasionally argued with each other, my English and communication

skills were greatly improved. We had fruitful collaborations that led to two chapters of this thesis. I thank Wei Shen, who initially proposed the idea on the work described in chapter 5. This project exposed me to a higher-dimensional thinking about materials. I would also like to express my heartfelt thanks to Rebecca Connor and Stacey Maskarinec for their proofreading and comments on my thesis. They helped me a lot in improving my writing in English. I enjoyed working with Shawn, Sunny and Mariam. Interactions with these undergraduate students gave me memorable tastes of mentoring. Also, discussions with other group members such as James Link, Tae Hyeon Yoo, Ayae Sugawara, Inchan Kwon, Charles Liu, Katherine Poulin, Kimberly Beatty, Jim Van Deventer, Eileen Fong, Nick Fisk, John Ngo, Caglar Tanrikulu, and Chihoon Ahn were very helpful.

Life at Caltech would be less enjoyable and colorful without my good friends here. Tingwei Mu was extremely helpful when I first arrived at Caltech. Friendship and support from Xiaoli Feng, Chengzhong Zhang, Zhipu Jin, Bolin Lin, Yougen Li, Robert Bao, etc. are memorable. It has been a lot of fun to play soccer and basketball with so many nice people. Those weekly games are very refreshing and keep me energetic.

Finally, I thank my family for encouraging me to pursue education and knowledge since my childhood. Without this driving force, I would not be able to finish the long journey from a small town in China to a PhD at Caltech. I truly thank my wife, Xiao Feng, for her deep love, for taking care of everything at home and putting up with my midnight working hours.

## ABSTRACT

Natural biomaterials are highly organized from the molecular to the macroscopic level in a hierarchical manner, requiring synthetic technologies to achieve this level of complexity. A biosynthetic approach to material design has emerged as an attractive option. In particular, proteins represent a promising class of molecules for creating new materials due to their determined sequence and structure. The research described in this thesis focuses on engineering protein-based materials using coiled-coil motifs. The coiled coil is a common protein architecture consisting of two or more  $\alpha$ -helices wrapped around one another to form a supercoil. Despite its simple conformation, the coiled-coil motif plays diverse roles in biological systems functioning as sensors, recognition elements, scaffolds, levers, rotating arms and springs.

First, a designed parallel heterodimeric leucine zipper pair was used as the protein capture domain to construct an artificial polypeptide scaffold for surface functionalization. By using a mutant *E. coli* phenylalanyl-tRNA synthetase, the photoreactive amino acid *para*-azidophenylalanine was incorporated. This protein polymer was spin-coated and photocrosslinked to octyltrichlorosilane-treated surfaces. The resulting protein films were shown to immobilize recombinant proteins through association of coiled coil heterodimer. Furthermore, in conjunction with microfluidic chips that were specifically designed for on-chip mixing using laminar flow, gradients of leucine zipper tagged proteins were formed in the microchannels and immobilized on the engineered protein films. This provides a general technique for producing surface-bound multicomponent gradients. The adhesion of human umbilical vein endothelial cells cultured on a surface-bound gradient

of cell binding ligands generated by this technique was examined. In addition, to generate protein walkers that have different lateral mobility rates on a surface, several variants of the leucine zipper pair with tunable heterodimerization affinities were designed and synthesized to allow diversity in the association strength of proteins linked to a surface.

The coiled-coil motif was also used to construct protein hydrogels. Hydrogels formed from a triblock artificial protein bearing dissimilar helical coiled-coil end domains (P and A) erode more than one hundred fold slower than hydrogels formed from those bearing the same end domains (either P or A). The reduced erosion rate is a consequence of the fact that looped chains are suppressed because P and A tend not to associate with each other. Thus, by harnessing selective molecular recognition, discrete aggregation number and orientational discrimination of coiled-coil protein domains, the erosion rate of hydrogels can be tuned over several orders of magnitude.

Finally, a biosynthetic approach was developed to control and probe cooperativity in multiunit biomotor assemblies by linking molecular motors to artificial protein scaffolds using the heterodimeric leucine zipper pair. This approach provides precise control over spatial and elastic coupling between motors. Cooperative interactions between monomeric kinesin-1 motors attached to protein scaffolds enhance hydrolysis activity and microtubule gliding velocity. However, these interactions are not influenced by changes in the elastic properties of the scaffold, distinguishing multimotor transport from that powered by unorganized monomeric motors. These results highlight the role of supramolecular architecture in determining mechanisms of collective transport.

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