ENGINEERING PROTEIN-BASED MATERIALS
THROUGH COILED-COIL MOTIFS

Thesis by
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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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