

ENGINEERING PROTEIN-BASED MATERIALS
THROUGH COILED-COIL MOTIFS

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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 α -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.

TABLE OF CONTENTS

Acknowledgements	iii
Abstract	v
Table of Contents.....	vii
List of Schemes and Tables.....	xi
List of Figures	xii
Chapter I. Introduction.....	I-1
1.1 Introduction to protein polymers	I-1
1.1.1 Background	I-1
1.1.2 Design and application of protein polymers	I-2
1.2 The coiled-coil motif.....	I-5
1.2.1 The structure of coiled coils	I-6
1.2.1 Model coiled-coil peptides	I-8
1.3 Thesis outline	I-12
1.4 References	I-12
Chapter II. Artificial Polypeptide Scaffold for Protein Immobilization	II-1
Abstract.....	II-1
2.1 Introduction	II-2
2.2 Results and discussion	II-3
2.3 Materials and methods	II-8
2.3.1 Cloning and expression of the polypeptide scaffold.....	II-8
2.3.2 Cloning and expression of fusion proteins.....	II-10

2.3.3 Protein array procedure.....	II-11
2.4 References	II-13

Chapter III. Generation of Surface-Bound Multicomponent

Gradients.	III-1
Abstract.....	III-1
3.1 Introduction	III-2
3.2 Materials and methods	III-4
3.2.1 Cloning, expression and purification of proteins	III-4
3.2.2 Surface functionalization	III-4
3.2.3 Fabrication of microfluidic chips	III-5
3.2.4 Quantitative measurement of surface density	III-6
3.2.5 Determination of dissociation kinetics.....	III-6
3.2.6 Cell culturing.....	III-6
3.2.7 Generation of immobilized protein gradients	III-7
3.2.8 Cell studies	III-8
3.3 Results and discussion	III-8
3.3.1 Design and biosynthesis of relevant proteins.....	III-8
3.3.2 Generation and characterization of immobilized protein gradients.....	III-10
3.3.3 Dissociation kinetics of immobilized proteins	III-15
3.3.4 Cellular response to surface-bound gradients	III-18
3.4 Conclusion and future work.....	III-20
3.5 References	III-20

Chapter IV. Progress towards the Creation of Protein Walkers IV-1

Abstract.....	IV-1
4.1 Introduction	IV-2
4.2 Materials and methods	IV-4
4.2.1 Cloning strategy	IV-4
4.2.2 Expression and purification of proteins.....	IV-6
4.2.3 Circular dichroism spectrometry	IV-7
4.2.4 Fluorescence recovery after photobleaching.....	IV-7
4.3 Results and discussion	IV-8
4.3.1 Design and characterization of heterodimeric leucine zipper pairs	IV-8
4.3.2 Synthesis of protein walkers and measurement of lateral mobility.....	IV-10
4.4 Conclusion.....	IV-14
4.5 References	IV-14

Chapter V. Tuning the Erosion Rate of Artificial Protein Hydrogels

through Control of Network Topology	V-1
Abstract.....	V-1
5.1 Introduction	V-2
5.2 Results and discussion	V-6
5.3 Materials and methods	V-17
5.4 References	V-19

Chapter VI. Engineering Cooperativity in**Biomotor-Protein Assemblies.....VI-1**

Abstract.....VI-1

6.1 IntroductionVI-2

6.2 Results and discussionVI-3

6.3 ReferencesVI-12

Appendix DAN sequence and plasmid mapsA-1

List of Schemes and Tables

<i>Number</i>		<i>Page</i>
Scheme 2.1	Surface functionalization and coiled-coil mediated immobilization of proteins	II-5
Scheme 3.1	The assembly of microfluidic device and gradient generation	III-12
Table 4.1	Amino acid sequences of synthesized leucine zipper variants	IV-4
Table 6.1	Summary of kinetics and temperature-dependent gliding assays	VI-6

List of Figures

<i>Number</i>	<i>Page</i>
Figure 1.1 Helical wheel representation of the parallel A1 homodimer	I-9
Figure 1.2 Helical wheel representation of the COMPcc pentamer	I-10
Figure 1.3 Schematic of the ZE/ZR heterodimer	I-11
Figure 2.1 Design of the artificial polypeptide scaffold 1 and related amino acid sequence	II-4
Figure 2.2 Immobilization and detection of proteins on polypeptide functionalized surfaces	II-7
Figure 2.3 Sequence modifications of the heterodimeric leucine zipper	II-8
Figure 2.4 Dicistronic expression construct for the polypeptide scaffold	II-9
Figure 2.5 Purification of ZE(gs) ₆ ELF by Ni-NTA affinity chromatography	II-10
Figure 2.6 Expression and purification of model proteins	II-11
Figure 3.1 SDS-PAGE of purified proteins	III-9
Figure 3.2 Calibration curve for quantification of ligand density	III-10
Figure 3.3 Fluorescence images of immobilized gradients and their corresponding density profiles	III-14
Figure 3.4 Dissociation curves of immobilized proteins monitored by changes in surface fluorescence	III-17
Figure 3.5 The attachment of HUVECs.	III-19
Figure 4.1 Cloning strategy for proteins containing multimeric leucine zipper variants	IV-5
Figure 4.2 SDS-PAGE analysis of purified RE ₃ R and RE ₂ RE peptides	IV-6
Figure 4.3 SDS-PAGE analysis showing expression and purification of proteins containing multimeric leucine zipper variants	IV-7

Figure 4.4	CD spectral data of RE ₂ RE, RE ₃ R, and ZE complexes to the basic ZR.	IV-9
Figure 4.5	Thermal denaturation curves of RE ₂ RE, RE ₃ R, and ZE complexes to ZR.	IV-10
Figure 4.6	A hypothetical model: proteins containing multimeric binding motifs “walk” across the surface through strand exchange.	IV-11
Figure 4.7	FRAP monitoring of protein walkers	IV-12
Figure 4.8	Spotting proteins on ZRELF surface and monitoring fluorescence change in PBS buffer after 11 hours.	IV-14
Figure 5.1	Schematic representations of triblock proteins and the amino acid sequences of major domains	V-3
Figure 5.2	Structural and dynamic properties underlying the fast erosion of AC ₁₀ A hydrogels	V-4
Figure 5.3	Coiled-coil domains A and P do not associate with each other	V-7
Figure 5.4	Dynamic moduli of hydrogels	V-8
Figure 5.5	Erosion profiles of hydrogels	V-10
Figure 5.6	The erosion profile of PC ₁₀ A hydrogels at 37 °C in Dulbecco's phosphate buffered saline	V-11
Figure 5.7	The erosion of PC ₁₀ A hydrogels at 37 °C in 3 ml of Dulbecco's modified eagle's medium (DMEM) with 10% fetal bovine serum added monitored over 4 days.	V-12
Figure 6.1	Engineered multimotor assemblies	VI-4
Figure 6.2	Enhancement of ATPase activity and motility when multiple K350-Z _E motors are coupled to (Z _R -ELF ₅) _n polymer scaffolds	VI-6
Figure 6.3	Influence of the condensation of the ELF domain of the polymers on multimotor motility.	VI-9