

# Force Spectroscopy of Stimulus-Responsive Polymers

Principal Investigator: Stefan Zauscher \*

Co-Pi: Ashutosh Chilkoti † and Robert Clark ‡\*

Center for Biologically Inspired Materials and Materials Systems  
Pratt School of Engineering, Duke University, Durham, NC 27708

Interactions on the level of single macromolecules become exceedingly important as we strive to understand and harness the properties of nanoscale systems. Specifically, we are interested in the nanomechanical behavior of stimulus-responsive polymers that display a strong volume transition which can be exploited for force generation. For example, the genetically encodable synthesis of stimulus responsive, elastin-like polypeptide (ELP) polymers was reported recently [1]. Upon increasing temperature, these protein-based polymers increase order by hydrophobic folding that results in an aggregated state and in the generation of useful work [2]. We potentially can harness the mechanical properties of these stimulus-responsive polymers and their ability to perform work by undergoing a reversible, inverse phase transition, to assemble devices on the nano- and meso-scale. These devices could encompass thermally activated clutches, clamps to immobilize cells, sensor surfaces, and pumps.

Bulk mechanical and physico-chemical properties are insufficient when we engineer structures on the nano- and meso-scales, because at these size scales the mechanical properties of an individual macromolecule will become important. Although single molecule force spectroscopy is a rapidly growing field (polysaccharides [3, 4, 5], synthetic polymers [6, 7, 8, 9], polypeptides [10, 11, 12, 13, 14, 15, 16, 17, 18]), measurement of the elastic behavior while a single, tethered ELP is undergoing an environmentally induced hydrophobic folding have not been reported.

The goal of the proposed research is therefore focused on studying the interplay between macromolecular structure –over which we have exquisite control through genetic engineering– and phase behavior and its effect on the elasticity and conformation of a single ELP chain. Concomitant with these measurements we also study the surface mechanical and adhesive properties of thin hydrogels formed by these stimulus-responsive polymers. By systematically varying the molecular structure of the polymers and observing concomitant changes in the mechanical and surface chemical properties will allow us to establish a causal link between the structure of the molecular building blocks and material properties.

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\*Department of Mechanical Engineering and Materials Science

†Department of Biomedical Engineering

‡Director of the Center for Biologically Inspired Materials and Material Systems

## Research Plan

**Surface Functionalization with ELP Chains** One concern in single molecule measurements is the anchor strength of the macromolecule to the substrate surface. Previous measurements indicate bond strengths for the covalent Si-C bond on the order of 2 nN, for the Au-S bond on the order of 1.4 nN, while non-specific adsorption leads to sub-nanonewton adhesion forces [19]. The forces associated with chain entropic elasticity are usually one order of magnitude smaller than the tethering strengths, however, depending on the forces generated during folding, covalent tethering may become necessary. We will immobilize ELP molecules by covalent attachment to carboxyl terminated, self-assembled monolayers on gold surfaces, or by covalent attachment to silanated siliceous surfaces. Suitable ELPs are molecularly engineered in Dr. Chilkoti's laboratory.

## Force Spectroscopy

We will operate the force spectrometer in displacement and force-feedback control mode. While displacement control is useful for molecule stretching, force control will be used to hold the molecule tethered between substrate and cantilever tip. This should be feasible as long as the instrumental drift is smaller than the changes in molecular length of interest; our instrument design addresses some of the stability concerns by implementing a closed-loop PZT actuator feedback control. In due course of the project, control algorithm development, design and construction of new hardware are an integral part. This work will be performed in close cooperation with the research group of Dr. Clark in the Center for Applied Control.

The ELP pentapeptide repeats are arranged into a folded  $\beta$ -helix with a pitch of 15 aminoacids. This structure suggests a description of the elastic behavior of a single chain by an extended worm-like chain (WLC) model. Fitting variables are usually the persistence length that determines the slope in the low force regime, and the segment elasticity that determines the slope in the high force regime [9]. Fits of the WLC model to force spectroscopy data are usually remarkably good, and deviations can be explained in terms of suprastructures caused by additional interactions among chain segments or with solvent molecules [9]. In case of ELPs we will analyze these deviations and interpret them as indicators for structural changes in the polymer, resulting from temperature and solvent induced changes in the unfolding pathway. To measure the forces associated with chain collapse, we tether the molecule at fixed extension and measure the force exerted on the cantilever spring while the temperature is changed through the LCST, or while the operating temperature of the ELP is lowered through addition of salt.

## Summary

Elastin like polypeptides are environment-responsive polymers that provide the exceptional advantage that their molecular structure can be precisely manipulated, making nanomechanical analysis with molecularly engineered constructs possible. Systematically varying the protein structure and observing concomitant changes in the mechanical properties of the assemblies will help to understand and tailor molecular properties of ELPs for specific

applications. Single molecule force spectroscopy, performed while an ELP molecule is undergoing a stimulus induced phase transition, will provide detailed information about the molecules' conformational changes during such a transition, and will give a direct measure of the forces generated during contraction and expansion. This information is necessary for effective polymer synthesis, and the rationale design of nano-scale devices using stimulus-responsive polymers. Applications for ELPs are presently studied by Dr. Chilkoti in the Department of Biomedical Engineering at Duke University.

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