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Single-molecule manipulation measurements of polymer/solution interactions

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Chapter 1

Introduction

1.1 Prologue: Stretching a Single Human Hair

The properties of polymer materials derive from physics at the single-molecule level. As an example, the stretching behavior of human hair is governed by structural changes in its constituent protein molecules. This is revealed in a simple mechanical test.

Our testing approach is to isolate a single hair and measure its change in length as a function of applied force. Sophistocated equipment is not necessary; constant and stable millinewton-scale forces can be applied using gravity, and length changes on the millimeter scale are easily measured with a ruler.

A simple way to perform this test is to clamp either end of the hair using small mounting hardware (a screw and nut). We use one of these clamps to also tie an inextensible thread (SpiderWire brand fishing line works well), which holds a weight bucket (a polystyrene cup) below the hair. Holding the hair by the upper

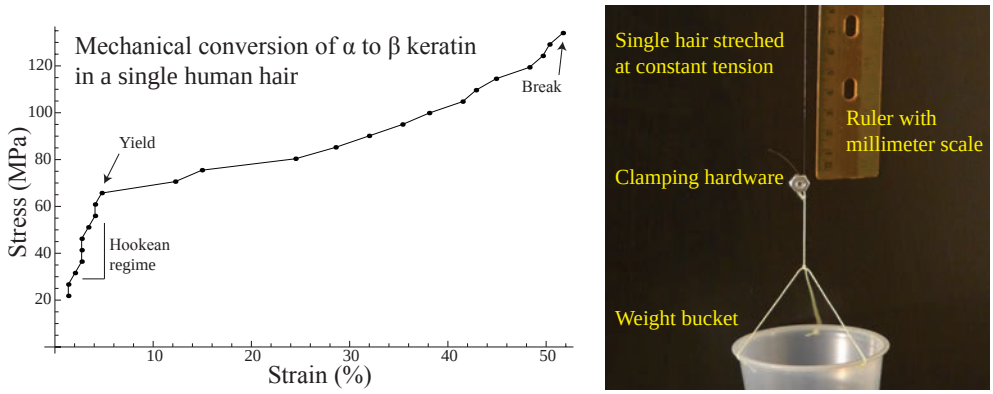

Figure 1.1: *Left* Stretching data of a single human hair. A resting length of 184 mm and a nominal diameter of 0.04 mm were used to normalize the data. The smallest load of 8.68 g included the weight of the hair, lower clamp, and bucket before adding pennies. *Right* Experimental geometry for stretching hair using gravity.

clamp, the combined weight of the hair, lower clamp, thread and bucket gives a small stretching force of less than 0.1 N. We measure the end-to-end length of hair between the clamps before adding weight to the bucket. We then slowly increase the weight in 2.5 g (one penny) increments while repeating the length mesurement and obtain the data shown in the figure.

The first thing to notice is that at low forces, the hair stretches as a Hookean spring. The stretching is not permanent in this elastic regime and the hair will immediately return to its original length after relieving the tension. However, over a narrow range of higher forces the hair begins to rapidly lengthen. The hair stiffens during this high-strain transition before finally breaking.

What causes this strange stretching behavior? The hair fiber contains a hierarchy of structure whose core is built of protein molecules [1]. These are natively structured as coiled-coils, and under force exhibit the superelastic stretching, energy dissipation, and strain hardening that make hair a tough material.

In fact, data such as those in the figure were key to the discovery of protein structure [2]. In the 1930s, experimentalist William Astbury was the first to recogize that the features of the mechanical response curve reflect a conformational change occuring in the protein molecules within the hair. He named the unstretched state α and the extended state β. These names were kept by Linus Pauling and coworkers when they correctly proposed the α-helix and β-sheet models of protein secondary structure in 1951.

Tensile testing is an especially powerful measurement approach because the product of force and extension is energy. The hair is stretched with forces measured in mN and lengths measured in mm; and the dissipated energy (area under the curve) is on the order of a tenth of a Joule. The mechanics of a single hair derive from the collective behavior of billions of protein molecules. For a single protein molecule, the relevant forces and lengths occur on the thermal energy scale, which at room temperature is k_BT=4.1 $\times$ 10^{-21} J=4.1 pN·nm. (Also, $k_BT = 9.83 \times 10^{22}$ cal = 0.0256 eV = 2.479 kJ/mol = 0.593 kcal/mol.) This immense change in magnitude underscores just how enormous a hair is compared to a single chain molecule. In analogy to stretching hair using gravity, we will follow a similar testing approach and stretch single polymers using constant magnetic forces; we will use force to manipulate the structure of a polymer and measure its physical properties in aqueous solvent.

1.2 Why Measure Single Molecules?

Direct polymer manipulation enables quantitative measurements with unprecedented precision. The approach permits access to detailed structural, thermodynamic, or even time-resolved behaviors that are otherwise obscured by ensemble averaging in traditional characterization techniques.

1.2.1 Mechanical Work is a Free Energy Term

Force and extension are thermodynamic conjugate variables. Since the product of force and extension is energy, simultaneous measurement of these variables provides access to the free energy of the system. Unlike the typical thermodynamic variables – such as pressure and volume, which act on the molecule through a global solution change – force and extension are *vectors*: Force acts directionally and specifically on the polymer being pulled, guiding a physical change while keeping the solvent (its temperature, pH, salinity, etc.) at a fixed condition.

1.2.2 Force Favors Extended States

We can gently guide the system using mechanical perturbation and pulling provides a natural reaction coordinate:

1. The free energy vs extension profile tilts in the direction of applied force, meaning that force favors extended conformations while destabilizing compact conformations.

2. At equilibrium, the thermodynamic change is equal to the applied mechanical work.

3. For a single kinetic barrier separating two thermodynamic wells, the consequence for force lowering the barrier is that the frequency of sampling the more extended state increases exponentially with the applied force.

1.2.3 Forces in Biology: Mechanics and Thermodynamics

Life is animated by connecting thermal and mechanical forces. Everyday actions such as lifting your arm are truly remarkable – being achieved through the coordinated performance of trillions of biomolecules that act as motors, ratcheting the muscle fibers into a flexed position. Cells divide, crawl, and remodel their supportive structures through the production of mechanical forces. At a molecular level, these forces are produced in the generation of strain that coincides with a conformational change in the molecule. The change in molecular structure is *thermally* driven, and facilitated and directed by the expenditure of chemical energy, such as in the hydrolysis of ATP.

In a biological context, mechanical forces have relevance. Cells sense their surroundings through mechanotransduction in addition to chemical signaling; for example, fibronectin regulates cellular adhesion with a force-dependent active site that works as a mechanical signal transducer [3]. A tensile force can open an ion channel to initiate cellular differentiation [4], or provide a means of communicating a signal across a cellular membrane [5]. Within the cell, transcription requires the double-helical DNA to be separated into two strands so that the genetic information can be read. The DNA strands are forced apart by motor proteins. Thus the application of an unzipping force to denature a molecule such as DNA may

be closer to the relevant biological situation than the common laboratory use of heat or chemicals.

Because life is driven by thermal forces, a deep understanding of biological activities at the single-molecule level requires detailed knowledge of (i) the interactions between the biomolecule and the buffeting solvent, and (ii) how such interactions are related to biomolecular structure. We therefore set out to develop a single-molecule manipulation approach for measuring these fundamental aspects of biopolymer folding and function.

1.3 Overview of the Dissertation

By connecting single-molecule manipulation data with theories from polymer physics and thermodynamics, we develop the measurement framework for extracting two properties of a polymer in solution: (1) The *excluded volume* is a pairwise interaction occuring between parts of the same chain molecule and is modulated by the character of the solvent. (2) The *counterion excess* in the vicinity of a charged polymer such as DNA mediates interactions with other biomolecules and allows two DNA strands to hybridize and fold into a stable double helix.

We use the magnetic tweezers technique to manipulate single chain molecules and measure polymer properties. In Chapter 2 we describe the components of the experimental apparatus and basic methods. Chapter 3 supplies polymer physics background and defines some of the more obscure terminology used in Chapter 4. In the first part of Chapter 4, we present measurements of excluded volume in a synthetic polymer (PEG), which we chose as simple model system because it

is charge neutral and soluble in water. We develop a phase diagram of polymer structure, and then use this diagram to compare a variety of polymers. This includes our measurements of a charged polymer (single-stranded DNA) and also a protein molecule in the unstructured state.

Structure formation (folding) involves a change in the solvent in the vicinity of the molecule. In Chapter 5 we present a measurement technique for probing changes in the local ion atmosphere upon folding of a DNA hairpin. By counting changes in ion excess, we precisely measure the electrostatic component of DNA stability and show that, contrary to the conventional interpretation, the extent of charge stabilization in DNA depends on the bulk concentration of monovalent salt. Our expermental results lead us to question the existing thermodynamic framework that has been widely used to interpret thermal DNA denaturation curves.

Although we establish new measurement tools in this work using rather simple model systems, the methods presented here are quite general and can be extended to a variety of systems. We conclude with a few recommendations for future research directions in Chapter 6. Finally, the appendices detail experimental procedures (including schemes for polymer immobilization and temperature control) that may be especially useful for practitioners of the magnetic tweezers technique.

Bibliography

[1] R. L. Akkermans and P. B. Warren. Multiscale modelling of human hair. *Philos Trans A Math Phys Eng Sci*, 362(1821):1783–93.

[2] D. Eisenberg. The discovery of the alpha-helix and beta-sheet, the principal structural features of proteins. *Proc Natl Acad Sci U S A*, 100(20):11207–10.

[3] A. Krammer, H. Lu, B. Isralewitz, K. Schulten, and V. Vogel. Forced unfolding of the fibronectin type iii module reveals a tensile molecular recognition switch. *Proc Natl Acad Sci U S A*, 96(4):1351–6.

[4] Spyros Artavanis-Tsakonas, Matthew D. Rand, and Robert J. Lake. Notch signaling: Cell fate control and signal integration in development. *Science*, 284(5415):770–776, 1999.

[5] C. R. Nol, J. Mazar, J. A. Melvin, J. A. Sexton, and P. A. Cotter. The prodomain of the Bordetella two-partner secretion pathway protein FhaB remains intracellular yet affects the conformation of the mature C-terminal domain. *Mol Microbiol*, 86(4):988–1006.

[6] K. C. Neuman and A. Nagy. Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nat. Methods*, 5(6):491–505, June 2008.

[7] N. Ribeck and O. A. Saleh. Multiplexed single-molecule measurements with magnetic tweezers. *Rev. Sci. Instrum.*, 79(9):094301, 2008.

[8] B. M. Lansdorp, S. J. Tabrizi, A. Dittmore, and O. A. Saleh. A high-speed magnetic tweezer beyond 10,000 frames per second. *Rev Sci Instrum*, 84(4):044301, 2013.

[9] A. Dittmore, D. B. McIntosh, S. Halliday, and O. A. Saleh. Single-molecule elasticity measurements of the onset of excluded volume in poly(ethylene glycol). *Phys Rev Lett*, 107(14):148301.