UNIVERSITÄT LEIPZIG

University of Leipzig

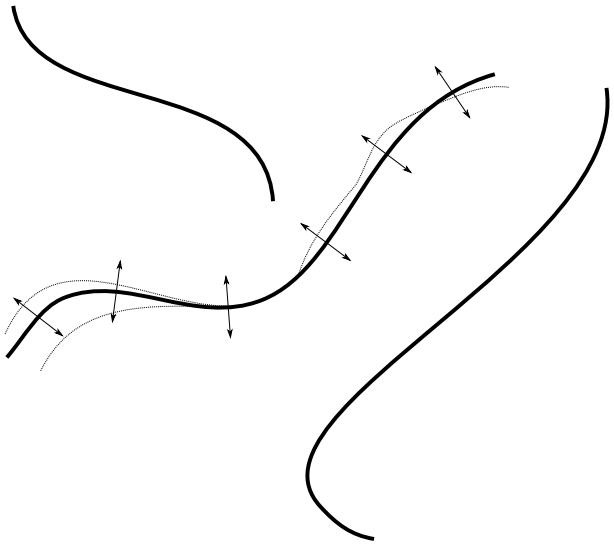
Faculty of Physics and Earth Science

Institute for Experimental Physics I

Department of Soft Matter Physics

Bending Stiffness of Actin Filaments

Soft Matter Physics Lecture - Experiment 2



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Chapter 1

Introduction

In general Soft Matter Physics covers matter that is small in size and interaction energy to be agitated by thermal motion at the environmental conditions. In particular Biological Physics is interested in such matter that is involved in living organisms. It turned out a variety of biological properties can be explained on the mesoscopic level by physical means. Characterizing the mechanical and some functional nature of cell leads to an accurate examination of the cytoskeleton.
This experiment's main aim is to visualize purified parts of the cytoskeleton and to determine one general physical quantity illustrating their polymer character.

1.1 The cytoskeleton

Three different families of highly conserved proteins constitute the cytoskeleton, namely microfilaments, microtubili and intermediate filaments. Every individual class features its own characteristic mechanical and dynamic properties but all of them obey some fundamental principles. Our grasp of the cytoskeleton bases on these principles detailed below and applies to the whole range of cells different in size, shape and motility.
In this practical course actin is studied as a biopolymer model system.

1.1.1 Actin

The actin monomer is an oblate globular (G-actin), roughly 42 kDa protein, conserved in all eukaryotic cells (cf. fig. 1.1). Its overall dimensions are $5.5\,\mathrm{nm} \times 5.5\,\mathrm{nm} \times 3.5\,\mathrm{nm}$, consisting of two domains, different in size, which comprise two subdomains each. The small domain's subdomain 1 exhibits the protein's C- and N-terminus. In the cleft in between, a divalent cation Mg^{2+} or Ca^{2+} is bound and complexed with the nucleotide ATP or ADP. The hydrolysis of ATP actin to ADP actin accompanies the assembly from actin monomers to the filamentous - polymer state.
The actin filament is a left-handed helix with a rise in length per monomer of 27.5 A and a rotation angle of $-166.2°$. Since this is close to $180°$ the filament appears as two right-handed long-pitch helices

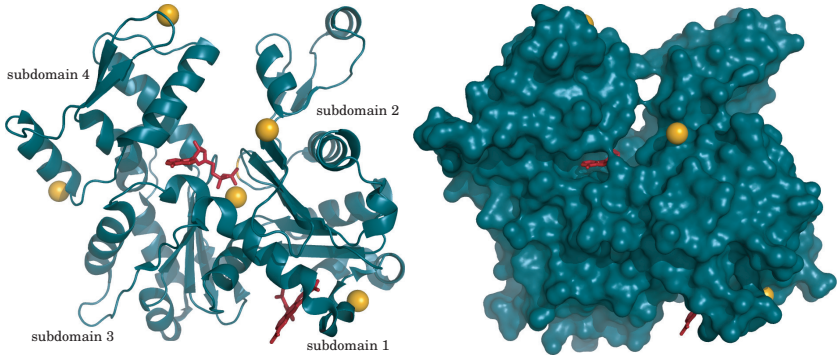

Figure 1.1: G-actin monomer. (**Left**) Ribbon representation. The light spheres depict the divalent cation Ca^{2+}. In the center, where all subunits are connected, an ADP nucleotide is bound with the primary Ca^{2+} associated. Additionally, a tetramethylrhodamine-5-maleimide fluorophore is covalently bound to the Cys 374 residue, indicated by the stick model at the subdomain 1. (**Right**) The same protein but in surface representation to visualize the building block character.

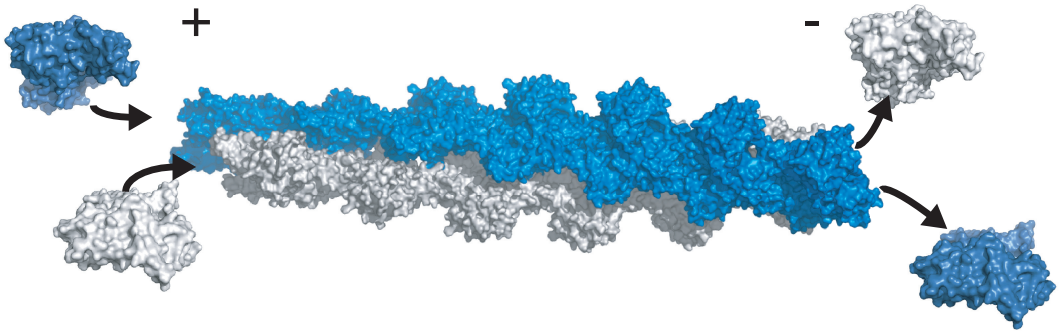

Figure 1.2: Actin filament assembled of 13 monomers. It is helical with 13 actin molecules per 6 left-handed turns a repeat of about 360 A. Since the rotation per molecule is 166°, the actin helix morphologically appears as two right-handed steep helices which twine slowly round each other, depicted by light and dark colored monomers (Holmes et al., 1990). Plus (barbed) and minus (pointed) end are indicated.

twisting around each other (cf. fig. 1.2). 7 nm to 9 nm in diameter the filament has the large actin domain next to the filament's axis and the small domain more peripheral. This structural conformation leads to a polarity of the filament with a barbed (+) and a pointed end (-). In the absence of actin-binding proteins, the exponential length distribution with a mean of approximately 7 µm is independent of actin's concentration (Sept et al., 1999). From a mechanical point of view, actin filaments are semi-flexible polymers with a persistence length of 18 µm stabilized with phalloidin and 9 µm unstabilized (Isambert et al., 1995).

Different proteins bind to actin and alter its structural function. They divide into several groups: actin binding proteins that bind to G-actin and hinder polymerization (e.g. profilin at the pointed end),

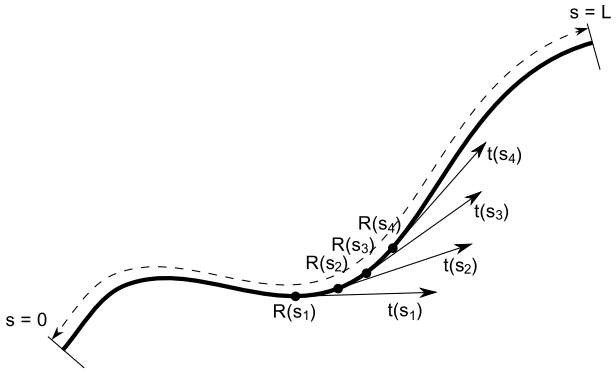

Figure 1.3: A semi-flexible polymer with an overall contour length L, the points of the filament $\boldsymbol{R}(s)$ with the parameter $s \in [0, L]$ and the unit tangent vector $\boldsymbol{t}(s)$.

proteins that diffuse to the filament's ends and cap them permanently (e.g. capZ), proteins that sever filaments (e.g. cofilin), proteins that bind laterally and stabilize filaments (e.g. tropomyosin), motor proteins that move along filaments (e.g. myosin) and proteins that connect filaments mutually either in networks (e.g. Arp2/3) or bundles (e.g. fascin) (Kabsch and Vandekerckhove, 1992). Some proteins provide a combination of these functions, e.g. gelsolin servers and caps filaments. The role of the highly conserved actin nucleator formin is not fully understood. It nucleates local filaments, facilitates processive capping, i.e. it elongates at the barbed end and prevents it from terminated capping.

1.1.2 The worm-like chain

Semi-flexible polymers are described by the worm-like chain model. In contrast to the freely-jointed chain, within the scope of this approach the polymer is assumed to be continously flexible, represented by a constant flexural rigidity EI. Calculating the energy referred to this, we consider the position of a point of the filament $\boldsymbol{R}(s)$ and the unit tangent vector

$$\boldsymbol{t}(s) = \frac{\partial \boldsymbol{R}(s)}{\partial s}, \quad |\boldsymbol{t}(s)| = 1, \tag{1.1}$$

where s is the parametrization of the polymer running from zero to the overall contour length L. All quantities are illustrated in figure 1.1.2
The elastic bending energy U has to be quadratic and adds up over the whole filament length

$$U = \frac{1}{2} EI \int_0^L \left(\frac{\partial \boldsymbol{t}}{\partial s} \right)^2 \mathrm{d}s. \tag{1.2}$$

Straightforward calculation (cf. (Doi and Edwards, 1986) or (Howard, 2001)) of the vector correlation function

$$\langle \boldsymbol{t}(s) \cdot \boldsymbol{t}(0) \rangle = \frac{1}{L} \int_0^L \boldsymbol{t}(s') \cdot \boldsymbol{t}(s' + s) \, \mathrm{d}s' \tag{1.3}$$

and application of the equipartition theorem yields

$$\langle \boldsymbol{t}(s) \cdot \boldsymbol{t}(0) \rangle = \begin{cases} e^{-\frac{s}{2l_p}}, & \text{if} \boldsymbol{R}, \boldsymbol{t} \in \mathbb{R}^2 \\ e^{-\frac{s}{l_p}}, & \text{if} \boldsymbol{R}, \boldsymbol{t} \in \mathbb{R}^3 \end{cases} \tag{1.4}$$

with the decay constant "persistence length"

$$l_p = \frac{k_\mathrm{B} T}{EI}. \tag{1.5}$$

The mean-squared end-to-end distance in three dimensions obeys

$$\left\langle \boldsymbol{R}^2 \right\rangle = 2l_p^2 \left\{ e^{-\frac{L}{l_p}} - 1 + \frac{L}{l_p} \right\}. \tag{1.6}$$

Chapter 2

Experiment

To accomplish the experiment an adequate theoretical background knowledge about the main topics is mandatory. Thus, questions may be ask to test the applicability of the experimenter. Furthermore, every student has to be familiar with the safety provisions.

2.1 Preview

For an adequate preparation the experimenter has to present the following points in the preview section:

- Actin
- Biopolymer models
- The worm-like chain model
- Equipartition theorem
- Autocorrelation function
- Fluorescence microscopy
- Confocal microscopy

2.2 Experimental procedure

The main task in the preparation part is to create an appropriate experiment to investigate single polymer bending in solution. Therefore, the curvature of suspended actin filaments on a glass slide should be observed.
For the accomplishment, the following equipment will be available:

- Fluorescence microscope

- Labeled and unlabeled actin
- Buffer solution at physiological actin polymerization conditions
- Glass slides applicable with the microscope
- Vacuum crease
- Distilled water
- Pipettes

2.3 Data analysis

The collected data should be used to determine the persistence length of actin. In order to gain accuracy, averaging over several filaments is to be necessary. Discussion of possible errors should be performed qualitatively and quantitavively, respectively.
Further instructions will be given during the experimental procedure.

Chapter 3

Safety provisions for workers

Some general rules concerning safety at work

- before start: design of experiments, preparation, check of used equipment and chemicals
- knowledge about potential danger of the chemicals and adequate precaution (see safety data sheets)
- wearing of appropriate protective clothing
- doors and windows must be closed during the experiments
- no food, no drink, no smoke
- freezers for chemicals are not for food
- keep the labs clean, dispose wrapping immediately
- experimental equipment is signed clearly (person in charge, time of experiments)
- after finishing the experiments cleaning up, disposing waste
- scalpels, cannulas and glass waste in the concerning signed boxes
- only authorized persons have entrance in the labs

Storage, transport and handling of chemicals

- store chemicals in the original package (clear labeling, safety and danger advice)
- when using other boxes attend to clear and durable labeling
- when decanting of chemicals use adequate device (funnels ,pipettes...)
- when chemicals toxic or corrosive use extractor hood

- no needless supply inventory of chemicals
- transport of chemicals (glass bottles) in racks or buckets - possible risk of breakage

Handling with liquid nitrogen

- wear safety glasses
- the filled liquid nitrogen tank must be transported in the elevator by oneself (risk of suffocation in emergency case!)

Some Special Rules S1 / S2 Labs

S1 and S2 are safety levels for genetic engineering labs and defined in the Gentechnikgesetz as:

S1 - no risk for human health and environment due to genetically modified organisms

S2 - minor risk for human health and environment due to genetically modified organisms

(the classification is resulted from Zentrale Kommission für biologische Sicherheit
http://www.bvl.bund.de/DE)
S1 labs are 131a/b (AFM), 309 (bong lab) and 310 (sample prep.) S2 labs are 116 (cell culture lab) and 311 (stretcher lab)

In principle

- apply the instructions on how to do genetical operations (S1/S2 level)
- follow the instructions of the hygiene plan

(both are published in every lab)

General

- wear protective clothing
- protective clothing must not be worn outside the labs
- workings of the following kind require nitril-protective gloves:
- passaging of transfected or virus-infected cell lines

- preparation with potentially infectious cells or tissue from animals and when dealing with human blood or tissue
- caustic, poisonous or mutagene material and material combinations
- mouth-pipetting is strictly prohibited
- syringes and canulaes should only be used if it cannot be avoided
- after every single working step or when leaving the S1/S2 lab, hands are to be washed and disinfected (see hygiene plan)
- disinfect work spaces after working with genetically modified organisms or pathogenic germs
- all equipment that had contact with genetically modified organisms has to be disinfected or has to be autoclaved
- lock the door when leaving the lab

Waste disposal

- collect contaminated material separately, non-fluid and liquid lab waste in different boxes
- waste that could contain genetically modified orgamisms or pathogenic germs must be autoclaved before disposing
- collect organic solvents and poisonous substances separately

In case of an accident

- if an accidental release of genetically modified organisms has happend, please inform all employees and superior authorities immediately (Prof. Käs, Undine)
- spilled biological material must be adsorbed at once and disinfected according to the rules of the hygiene plan
- if an extensive contamination of equipment or working spaces cannot be avoided, please turn off the device, ensure that nobody gets close to the site of accident and decontaminate the space carefully
- contaminated protective clothing must be taken off and be put into the autoclave
- contaminated skin must be medicated with a special disinfectant
- injuries should bleed properly (give off dangerous substances)
- after contamination of the mucous membranes - clean with water (eye shower room 310, 178)
- every injury / accident has to be register

Disinfectants

- equipment: Descosept, 80% ethyl alcohol
- tables: Descosept, 80% ethyl alcohol
- hands: Sterilum

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