

Contents

1	Introduction	1
	References	4
2	State of the Art	5
2.1	Intermediate Filaments	5
2.1.1	Architecture of Intermediate Filaments	6
2.1.2	Assembly of Intermediate Filaments	7
2.1.3	Properties of Intermediate Filaments – Similar, but not Identical	9
2.2	Intermediate Filaments under Load	10
2.2.1	Mechanics of Individual Intermediate Filaments	11
2.2.2	Simulations of Intermediate Filament Mechanics	13
2.2.3	Intermediate Filaments and the Mechanical Properties of Cells.	14
2.3	Mathematical Models for Biopolymers	17
2.3.1	Freely-jointed Chain and Worm-like Chain	17
2.3.2	Entropic Springs	21
2.3.3	Two-State Model	22
2.4	Optical Tweezers.	24
2.4.1	Physics of Optical Trapping	25
2.4.2	Calibration of Optical Traps.	27
	References	31
3	Materials and Methods	41
3.1	Vimentin expression and purification	41

3.2	Labeling of Vimentin	44
3.2.1	Labeling with Fluorescent Dyes	45
3.2.2	Labeling with Biotin	46
3.3	Vimentin Reconstitution and Assembly	47
3.4	Maleimide-functionalization of Beads	48
3.5	Optical Tweezers Experiments	48
3.5.1	Setup	49
3.5.2	Calibration of Optical Traps.	49
3.5.3	Sample Preparation	50
3.5.4	Measurement Preparation and Basic Experimental Procedure	51
3.5.5	Measuring Procedures	52
3.5.6	Cleaning Protocol for the Flow Cell	55
3.6	Data Processing	56
3.6.1	Processing for further analysis	58
3.6.2	Processing Force-Distance Curves	59
3.6.3	Processing of Force Clamp and Creep Data	60
3.6.4	Correction of Force Offset	60
	References	64
4	Nonlinear Loading-Rate- Dependent Force Response of Individual Vimentin Intermediate Filaments to Applied Strain	65
4.1	Abstract	67
4.2	Introduction	67
4.3	Materials and Methods	69
4.4	Results	70
4.5	Discussion.	75
4.6	Supplementary Information.	78
4.6.1	Theoretical model	78
4.6.2	Comparison of Worm Like and Freely Jointed Chains	79
4.6.3	Fit Results.	80
	References	83

5	Viscoelastic Properties of Vimentin Originate from Nonequilibrium Conformational Changes	87
5.1	Abstract	89
5.2	Introduction	89
5.3	Results	91
5.3.1	Pronounced Energy Dissipation	91
5.3.2	A Biopolymer with Tensile Memory	93
5.3.3	Two Modes of Filament Elongation	95
5.4	Discussion.	97
5.5	Material and Methods	100
5.5.1	Vimentin Expression and Purification Protocol	100
5.5.2	Vimentin Labeling	102
5.5.3	Vimentin Reconstruction and Assembly	102
5.5.4	Maleimide Functionalization of Beads	103
5.5.5	Experimental setup and procedure	103
5.5.6	Analysis of Optical Tweezers Data.	104
5.6	Models	105
5.6.1	Effective Two-State Model	105
5.6.2	Analytical Solution for FC Conditions	108
5.6.3	MC Simulation of Unfolding and Refolding of Parallel Elements	108
5.7	Supplementary Materials.	113
5.7.1	Movie S1	118
	References	121
6	Mechanical Response of Glutaraldehyde Stabilized Vimentin IFs	125
6.1	Introduction	125
6.2	Loading-Rate-Dependency	127
6.3	Creep Behavior at Constant Force	131
6.4	Discussion.	132
	References	134
7	Summary, Discussion and Conclusion	137
	References	143

List of Publications	145
Lebenslauf	147
Danksagung	149
A Supplementary Information – Energy Dissipation	153