

Contents

1	Introduction to DNA	1
1.1	Introduction	1
1.2	Single-molecule versus bulk studies	2
1.3	DNA structures	3
2	Manipulating DNA	11
2.1	Tailoring and labelling DNA	11
2.2	Molecular combing	12
2.3	Forces at the molecular scale	15
2.4	Micromanipulation tools	15
2.5	Choosing a single-molecule manipulation tool	22
2.6	Problems	26
3	Fluorescence Spectroscopy and Microscopy Techniques	27
3.1	Introduction	28
3.2	Principles of fluorescence	32
3.3	Illumination methods	49
3.4	Detection techniques	55
3.5	Single-molecule tracking	58
3.6	Perspectives	62
3.7	Problems	63
4	The Mechanical Properties of Nucleic Acids	67
4.1	Models of stretched polymers	69
4.2	Relaxation dynamics of DNA	74
4.3	The elastic behaviour of twisted DNA	76
4.4	Torque estimate from single-molecule thermodynamics	82
4.5	Twist-stretch coupling in DNA	86
4.6	The braiding of two DNA molecules	88
4.7	Simulations of stretched, twisted, or braided molecules	90
4.8	Single-stranded nucleic acids	94
4.9	Pulling on RNA	98
4.10	Problems	103
5	Structural Transitions in DNA	105
5.1	Molecular transitions induced by twisting	105
5.2	Molecular transitions induced by stretching	110

5.3 Unzipping DNA	113
5.4 Conclusions and perspectives	116
6 Single-Molecule Studies of DNA-Associated Motors	119
6.1 Introduction to DNA-protein interactions	119
7 DNA and RNA Polymerases	121
7.1 Introduction to polymerases	121
7.2 An introduction to replication	121
7.3 Single-molecule <i>in vitro</i> DNAP experiments	123
7.4 <i>In vivo</i> studies of single replication complexes	132
7.5 An introduction to transcription	133
7.6 Single-molecule <i>in vitro</i> RNAP experiments	139
7.7 <i>In vivo</i> studies of transcription	151
7.8 Conclusions and perspectives	153
8 DNA Helicases	155
8.1 Introduction to helicases	155
8.2 Bulk versus single-molecule assays: the case of RecBCD	158
8.3 Active and passive unwinding mechanisms: RecQ versus gp41	160
8.4 The UvrD helicase studied by single-molecule manipulations	164
8.5 Single-molecule FRET studies of the Rep helicase	171
8.6 Problems	174
9 Topoisomerases	177
9.1 Introduction to topoisomerases	177
9.2 Bulk assays	180
9.3 Single-molecule <i>in vitro</i> assays: type I topoisomerases	181
9.4 Single-molecule <i>in vitro</i> assays: type II topoisomerases	185
9.5 Single-molecule study of the coiling activity of gyrases	195
9.6 <i>In vivo</i> studies of single topoIV localization and action in <i>E.coli</i>	197
10 Conclusions	199
References	201
Index	223