Mechanics of Motor Proteins and the Cytoskeleton

Jonathon Howard

Department of Physiology and Biophysics
University of Washington
and
Max Planck Institute for Molecular Cell Biology and Genetics
# Brief Contents

1 Introduction 1

**PART I Physical Principles 7**

2 Mechanical Forces 9  
3 Mass, Stiffness, and Damping of Proteins 29  
4 Thermal Forces and Diffusion 49  
5 Chemical Forces 75  
6 Polymer Mechanics 99

**PART II Cytoskeleton 117**

7 Structures of Cytoskeletal Filaments 119  
8 Mechanics of the Cytoskeleton 135  
9 Polymerization of Cytoskeletal Filaments 151  
10 Force Generation by Cytoskeletal Filaments 165  
11 Active Polymerization 179

**PART III Motor Proteins 195**

12 Structures of Motor Proteins 197  
13 Speeds of Motors 213  
14 ATP Hydrolysis 229  
15 Steps and Forces 245  
16 Motility Models: From Crossbridges to Motion 263

Afterword 285  
Appendix 287  
Bibliography 339  
Index 361
Contents

1 Introduction 1

PART I Physical Principles 7

2 Mechanical Forces 9

Force 10

EXAMPLE 2.1 PHYSICAL FORCES AND THEIR MAGNITUDES AT THE SINGLE-MOLECULE LEVEL 12

Motion of Springs, Dashpots, and MASSES INDUCED BY APPLIED FORCES 14

EXAMPLE 2.2 THE FORCE GENERATED BY THE BACTERIAL MOTOR 16

Motion of Combinations of Mechanical Elements 16

EXAMPLE 2.3 THE INERTIA OF A BACTERIUM 18

EXAMPLE 2.4 THE PERSISTENCE OF PROTEIN MOVEMENTS 18

EXAMPLE 2.5 THE TIMESCALE OF PROTEIN CONFORMATIONAL CHANGES 19

EXAMPLE 2.6 VIBRATION OF CHEMICAL BONDS 21

EXAMPLE 2.7 PROTEIN VIBRATIONS 22

Motion of a Mass and Spring with Damping 22

EXAMPLE 2.8 MOTOR PROTEINS ARE OVERDAMPED 24

Work, Energy, and Heat 24

EXAMPLE 2.9 ENERGY OF CHEMICAL BONDS 25

EXAMPLE 2.10 ENERGY STORED IN PROTEIN CONFORMATIONAL CHANGES 26

Summary: Generalizations to More Complex Mechanical Systems 26

Problems 26

3 Mass, Stiffness, and Damping of Proteins 29

Mass 29

Elasticity 30

EXAMPLE 3.1 STIFFNESS OF A ROD UNDER TENSION 32

EXAMPLE 3.2 THE CANTILEVER SPRING 32

EXAMPLE 3.3 THE COILED SPRING 33

The Molecular Basis of Elasticity 34

EXAMPLE 3.4 THE YOUNG’S MODULUS OF A COVALENT SOLID 35

Viscous Damping 37

EXAMPLE 3.5 JAR OF HONEY 39

The Molecular Basis of Viscosity 39

The Global Motions of Proteins are Overdamped 41

EXAMPLE 3.6 RIBOSOME 43

The Motions of the Cytoskeleton and Cells ARE Also Overdamped 44

Summary 45

Problems 46
4 Thermal Forces and Diffusion 49

Boltzmann’s Law 50

EXAMPLE 4.1 APPLICATIONS OF BOLTZMANN’S LAW 52

Equipartition of Energy 53

Diffusion as a Random Walk 55

Einstein Relation 58

EXAMPLE 4.2 DIFFUSION OF IONS 59

Some Solutions to the Diffusion Equation 59

Free Diffusion from a Point Source 60

EXAMPLE 4.3 THE EFFICIENCY OF DIFFUSION AS A CELLULAR TRANSPORT MECHANISM 60

First-Passage Times 61

EXAMPLE 4.4 DIFFUSION OVER MOLECULAR DIMENSIONS 62

Correlation Times* 63

EXAMPLE 4.5 DIFFUSION OF A FREE PROTEIN 65

EXAMPLE 4.6 DIFFUSION OF A TETHERED PROTEIN 66

Fourier Analysis* 66

EXAMPLE 4.7 POWER SPECTRUM OF A DAMPED SPRING 69

The Magnitude of the Thermal Force* 69

EXAMPLE 4.8 THERMAL NOISE ALSO OCCURS IN ELECTRICAL CIRCUITS 70

Summary 71

Problems 71

5 Chemical Forces 75

Chemical Equilibria 76

The Effect of Force on Chemical Equilibria 78

EXAMPLE 5.1 MECHANICALLY SENSITIVE ION CHANNELS IN HAIR CELLS 80

Rate Theories of Chemical Reactions 83

Effect of Force on Chemical Rate Constants 88

EXAMPLE 5.2 THERMAL RATCHET MODELS FOR MOTOR PROTEINS 89

EXAMPLE 5.3 UNFOLDING TITIN USING AN ATOMIC FORCE MICROSCOPE 91

Bimolecular Reactions 92

Association Rates 92

Michaelis-Menten Equation 93

Protein Complexes 94

Cyclic Reactions and Free Energy Transduction 94

Summary 97

Problems 98

6 Polymer Mechanics 99

Flexural Rigidity and the Beam Equation 100

Applications of the Beam Equation: Bending and Buckling 102

The Cantilevered Beam 103

EXAMPLE 6.1 A GLASS FIBER 103

EXAMPLE 6.2 A MICROTUBULE 104

EXAMPLE 6.3 A COILED COIL 104

Buckling 104

EXAMPLE 6.4 MOTOR FORCE REQUIRED TO BUCKLE A MICROTUBULE 104

Drag Forces on Slender Rods 105

EXAMPLE 6.5 DRAG ON A SPERM 106

EXAMPLE 6.6 DRAG FORCES IN GLIDING ASSAYS 106

Dynamics of Bending and Buckling 106

EXAMPLE 6.7 RELAXATION OF MICROTUBULES AND ACTIN FILAMENTS 109

EXAMPLE 6.8 TIME CONSTANT OF A FORCE FIBER 109

EXAMPLE 6.9 DYNAMICS OF BUCKLING 109

Thermal Bending of Filaments 110

Persistence Length 110

Thermal Bending of Semiflexible Polymers 111

Entropic Elasticity of a Freely Jointed Chain 112

EXAMPLE 6.10 THE SPRING CONSTANT OF A FREELY JOINTED CHAIN 114

Worm-Like Chain 114

Summary 115

*An asterisk next to a heading denotes a more advanced section.
PART II Cytoskeleton 117

Problems 115

7 Structures of Cytoskeletal Filaments 119
Structures of the Subunits 121
Families of Cytoskeletal Proteins 122
  Actin 124
  Tubulin 124
  Intermediate Filament Proteins 124
Filament Structures 125
  Actin Filament 125
  Microtubule 126
  Coiled Coils 130
  Intermediate Filaments 131
Summary: Structural Basis for the Length, Strength, Straightness, and Polarity of Filaments 132
  Length 132
  Strength 133
  Straightness 133
  Polarity 133

8 Mechanics of the Cytoskeleton 135
Rigidity of Filaments in Vivo 136
  Longitudinal Stiffness of Actin in Muscle 136
  Bending Stiffness of Actin in Stereocilia 138
  Bending Stiffness of Microtubules in Sperm 140
  Rigidity of Keratin-Containing Materials 142
Rigidity of Filaments in Vitro 143
  Actin Filaments 143
  Microtubules 144
  Coiled Coils 145
  Intermediate Filaments 146
  Bacterial Flagellum 146
  DNA and Titin 147

Summary: Material Properties of Cytoskeletal Proteins 147

9 Polymerization of Cytoskeletal Filaments 151
Passive Polymerization: The Equilibrium Polymer 152
Single-Stranded Filaments Are Short 154
Multistranded Filaments Are Long 155
Multistranded Filaments Grow and Shrink at Their Ends 156
Other Properties of Multistranded Filaments 158
Binding Energies and the Loss of Entropy 159
Summary: Material Properties of Cytoskeletal Proteins 161
Structure and Dimensionality 161
Summary 162
Problems 162

10 Force Generation by Cytoskeletal Filaments 165
Generation of Force by Polymerization and Depolymerization in Vivo 166
Generation of Force by Polymerization and Depolymerization in Vitro 169
Equilibrium Force 169
Brownian Ratchet Model 172
  Reaction-Limited Polymerization 173
  Diffusion-Limited Polymerization 173
Examples of Motility Driven by Actin Polymerization 174
  EXAMPLE 10.1 ACTIN POLYMERIZATION IS FAST ENOUGH AND POWERFUL ENOUGH TO DRIVE THE MOVEMENT OF LISTERIA 175
  EXAMPLE 10.2 THE DIFFUSION-LIMITED SPEED OF LISTERIA 175
  EXAMPLE 10.3 ACTIN POLYMERIZATION IS FAST ENOUGH IN VITRO TO ACCOUNT FOR
### CONTENTS

**PART II Polymers 185**

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Work during Polymerization and Depolymerization 188</td>
</tr>
<tr>
<td></td>
<td>Structural Changes Attending Nucleotide Hydrolysis 191</td>
</tr>
<tr>
<td></td>
<td>Summary 193</td>
</tr>
</tbody>
</table>

**PART III Motor Proteins 195**

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Structures of Motor Proteins 197</td>
</tr>
<tr>
<td></td>
<td>Crossbridges and the Domain Organization of Motor Proteins 198</td>
</tr>
<tr>
<td></td>
<td>Motor Families 202</td>
</tr>
<tr>
<td></td>
<td>High-Resolution Structures 205</td>
</tr>
<tr>
<td></td>
<td>Docking of Motors to Their Filaments 210</td>
</tr>
<tr>
<td></td>
<td>Summary 212</td>
</tr>
<tr>
<td>13</td>
<td>Speeds of Motors 213</td>
</tr>
<tr>
<td></td>
<td>The Speeds of Motors in Vivo 213</td>
</tr>
<tr>
<td></td>
<td>Rowers and Porters 216</td>
</tr>
<tr>
<td></td>
<td>In Vitro Motility Assays 216</td>
</tr>
<tr>
<td></td>
<td>Processive and Nonprocessive Motors 219</td>
</tr>
<tr>
<td></td>
<td>The Hydrolysis Cycle and the Duty Ratio 221</td>
</tr>
<tr>
<td></td>
<td>Analogies to Internal Combustion Machines and Animal Locomotion 226</td>
</tr>
<tr>
<td></td>
<td>Summary 227</td>
</tr>
<tr>
<td>14</td>
<td>ATP Hydrolysis 229</td>
</tr>
<tr>
<td></td>
<td>ATP 229</td>
</tr>
<tr>
<td></td>
<td>Coupling Chemical Changes to Conformational Changes 232</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis of ATP by Skeletal Muscle Myosin 232</td>
</tr>
<tr>
<td></td>
<td>Without Actin 233</td>
</tr>
<tr>
<td></td>
<td>With Actin 234</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis of ATP by Conventional Kinesin 238</td>
</tr>
<tr>
<td></td>
<td>Without Microtubules 239</td>
</tr>
<tr>
<td></td>
<td>With Microtubules 239</td>
</tr>
<tr>
<td></td>
<td>Coordination of the Heads 239</td>
</tr>
<tr>
<td></td>
<td>Functional Differences between Kinesin and Myosin ATPase Cycles 242</td>
</tr>
<tr>
<td></td>
<td>Kinesin Is Attached during Its Rate-Limiting Step, but Myosin Is Detached 242</td>
</tr>
<tr>
<td></td>
<td>Biochemical Evidence for Kinesin’s Processivity 243</td>
</tr>
<tr>
<td></td>
<td>Biochemical Evidence that Myosin Has a Low Duty Ratio 243</td>
</tr>
<tr>
<td></td>
<td>Summary 244</td>
</tr>
</tbody>
</table>

**THE SPEED OF THE ACROSOMAL REACTION IN SEA CUCUMBER SPERM 176**

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Active Polymerization 179</td>
</tr>
<tr>
<td></td>
<td>Actin and Tubulin Hydrolysis Cycles 180</td>
</tr>
<tr>
<td></td>
<td>Summary 178</td>
</tr>
<tr>
<td>12</td>
<td>Other Kinetic Models 176</td>
</tr>
<tr>
<td></td>
<td>Summary 178</td>
</tr>
</tbody>
</table>

**PART III Motor Proteins 195**

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Structures of Motor Proteins 197</td>
</tr>
<tr>
<td></td>
<td>Crossbridges and the Domain Organization of Motor Proteins 198</td>
</tr>
<tr>
<td></td>
<td>Motor Families 202</td>
</tr>
<tr>
<td></td>
<td>High-Resolution Structures 205</td>
</tr>
<tr>
<td></td>
<td>Docking of Motors to Their Filaments 210</td>
</tr>
<tr>
<td></td>
<td>Summary 212</td>
</tr>
<tr>
<td>13</td>
<td>Speeds of Motors 213</td>
</tr>
<tr>
<td></td>
<td>The Speeds of Motors in Vivo 213</td>
</tr>
<tr>
<td></td>
<td>Rowers and Porters 216</td>
</tr>
<tr>
<td></td>
<td>In Vitro Motility Assays 216</td>
</tr>
<tr>
<td></td>
<td>Processive and Nonprocessive Motors 219</td>
</tr>
<tr>
<td></td>
<td>The Hydrolysis Cycle and the Duty Ratio 221</td>
</tr>
<tr>
<td></td>
<td>Analogies to Internal Combustion Machines and Animal Locomotion 226</td>
</tr>
<tr>
<td></td>
<td>Summary 227</td>
</tr>
<tr>
<td>14</td>
<td>ATP Hydrolysis 229</td>
</tr>
<tr>
<td></td>
<td>ATP 229</td>
</tr>
<tr>
<td></td>
<td>Coupling Chemical Changes to Conformational Changes 232</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis of ATP by Skeletal Muscle Myosin 232</td>
</tr>
<tr>
<td></td>
<td>Without Actin 233</td>
</tr>
<tr>
<td></td>
<td>With Actin 234</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis of ATP by Conventional Kinesin 238</td>
</tr>
<tr>
<td></td>
<td>Without Microtubules 239</td>
</tr>
<tr>
<td></td>
<td>With Microtubules 239</td>
</tr>
<tr>
<td></td>
<td>Coordination of the Heads 239</td>
</tr>
<tr>
<td></td>
<td>Functional Differences between Kinesin and Myosin ATPase Cycles 242</td>
</tr>
<tr>
<td></td>
<td>Kinesin Is Attached during Its Rate-Limiting Step, but Myosin Is Detached 242</td>
</tr>
<tr>
<td></td>
<td>Biochemical Evidence for Kinesin’s Processivity 243</td>
</tr>
<tr>
<td></td>
<td>Biochemical Evidence that Myosin Has a Low Duty Ratio 243</td>
</tr>
<tr>
<td></td>
<td>Summary 244</td>
</tr>
</tbody>
</table>

© Sinauer Associates, Inc. This material cannot be copied, reproduced, manufactured or disseminated in any form without express written permission from the publisher.
15 Steps and Forces 245

Distances that Characterize a Motor Reaction 245
Single-Motor Techniques 246
Forces 247
Displacements 250
EXAMPLE 15.1 SENSITIVITY OF A PHOTODETECTOR 250 Calibration 251
Single-Molecule Fluorescence 251
Steps, Paths, and Forces 251
Conventional Kinesin 252
Myosin II 255
Other Motors 259
The Structural Basis for the Duty Ratio 261
Summary 262

16 Motility Models: From Crossbridges to Motion 263

Macroscopic and Microscopic Descriptions of Motility 263
Powerstroke Model 265
Role of Thermal Fluctuations in the Power Stroke 268
Crossbridge Model for Muscle Contraction 269
Comparison of the Model to Muscle Data 273
Force-Velocity Curve and the Efficiency of Muscle Contraction 273
Mechanical Transients 276
Powerstroke Distance, Path Distance, and the Duty Ratio 277
One Step per ATP 279
Conclusion 279
A Crossbridge Model for Kinesin 280
Summary: Comparison between Motile Systems 282

Afterword 285
Appendix 287
Bibliography 339
Index 361