## Contents

Abstract vii

Contents ix

1 Introduction: The eukaryotic cytoskeleton and actin-microtubule coordination 1
  1.1 The eukaryotic cytoskeleton ........................ 1
  1.2 Cytoskeletal interactions .......................... 19
  1.3 Multiple roles for the cytoskeletal coordination toolbox .......... 32
  1.4 Motivation and thesis outline ....................... 32

2 General experimental methods 35
  2.1 Introduction ..................................... 35
  2.2 Flow cell preparation and surface functionalization .......... 35
  2.3 Buffer conditions to work with actin filaments and dynamic microtubules 39
  2.4 Microtubule polymerization and tip tracking assays .......... 41
  2.5 Proteins used in this thesis ........................ 42
  2.6 Buffers and stocks ................................ 44
  2.7 Total internal reflection fluorescence (TIRF) microscopy ........ 45
  2.8 Data analysis .................................... 46

3 TipAct – An engineered actin-binding microtubule +TIP 49
  3.1 Introduction ....................................... 49
  3.2 TipAct localization in mammalian cultured cells ............. 52
  3.3 In vitro characterization of TipAct ........................ 53
  3.4 Discussion ......................................... 60
  3.5 Materials and methods ................................ 62
  3.6 Data analysis ....................................... 68

4 Guidance of microtubule growth and organization by F-actin 69
  4.1 Introduction ......................................... 69
  4.2 TipAct and EB3 couple microtubule growth to F-actin bundles ... 71
  4.3 EB3 and TipAct have reduced off-rates at actin-microtubule overlaps . 73
4.4 Actin bundles capture and redirect growing microtubules ........ 76
4.5 Ordered arrays of F-actin bundles can globally dictate microtubule organization ................................... 84
4.6 Discussion .................................. 88
4.7 Materials and methods ................................ 91
4.8 Data analysis ................................ 92

5 F-actin organization by dynamic microtubules 103
5.1 Introduction .................................. 103
5.2 Growing microtubules deform and reposition F-actin bundles .... 105
5.3 Growing microtubules exert forces on single actin filaments .... 106
5.4 Growing microtubules organize F-actin networks .................. 108
5.5 Closing the loop: growing microtubules induce F-actin bundling .... 110
5.6 Discussion .................................. 111
5.7 Materials and methods ................................ 113
5.8 Data analysis ................................ 114

6 Transport and force generation by microtubule +TIPs 115
6.1 Introduction .................................. 115
6.2 Model of biased actin filament diffusion at microtubule tips .... 117
6.3 Gillespie-based simulations of actin filament transport by growing microtubules ................................... 135
6.4 Simulation results: effects of variable actin filament length, EB and tubulin concentrations ........ 137
6.5 Comparison between simulation and experimental data ........ 143
6.6 Further predictions of the model ................................ 146
6.7 Discussion .................................. 149
6.8 Materials and methods ................................ 154
6.9 Data analysis ................................ 154

7 Conclusions and outlook 159

Bibliography 163

Samenvatting 191

List of publications 195

Acknowledgements 197