Contents

1. Introduction to NF-κB signalling **1**

1.1. NF-κB . . . 2
1.2. Inhibitors of NF-κB . . . 3
1.2.1. IκBα . . . 3
1.2.2. IκBβ . . . 4
1.2.3. IκBϵ . . . 4
1.3. IκB kinase complex . . . 5
1.4. Zinc finger protein A20 . . . 6
1.5. Mathematical models of NF-κB signal transduction . . . 7

2. Methods **11**

2.1. Characterisation of dynamical behaviour . . . 11
2.1.1. Steady state and stability . . . 11
2.1.2. Characteristic time . . . 12
2.1.3. Deactivation time . . . 13
2.1.4. Maximal concentration . . . 14
2.1.5. Characteristic activation . . . 14
2.2. Sensitivity analysis . . . 15
2.3. Bifurcation analysis . . . 16
2.3.1. 1-parameter Hopf bifurcation analysis . . . 16
2.3.2. 2-parameter Hopf bifurcation analysis . . . 16
2.4. Data quantification . . . 17
2.5. Parameter estimation . . . 17
2.5.1. Parameter sampling . . . 18
2.5.2. Error model . . . 18
2.5.3. Maximum likelihood estimation . . . 19

I. Investigating different modes of dynamical behaviour in the canonical NF-κB pathway **21**

3. Modes of dynamical behaviour in mouse embryonic fibroblasts **23**

3.1. Experimental observations of NF-κB dynamics . . . 23
3.2. Model Reduction . . . 25
3.3. Characterisation of the core model of the canonical NF-κB pathway . 30
3.4. Identification of parameters that alter the dynamical profile of NF-κB 33
3.5. Verification of the model insights . . . 36

3.6. The total NF-κB concentration and the IκBα transcription rate constant influence the fold change of active NF-κB 39
3.7. The impact of external stimulation on the dynamics of NF-κB 40
3.8. Discussion 44

II. Feedback modulation in the canonical NF-κB pathway by post-transcriptional regulation of IκBα and A20 47

4. Feedback modulation in human embryonic kidney cells 49
4.1. Motivation 49
4.2. Experimental data 50
4.3. Mathematical model 52
4.4. Influence of RC3H1 overexpression and knock-down on NF-κB activation 54
4.5. Experimental validation of model predictions of RC3H1 effects on NF-κB 57
4.6. Dissecting the effect of RC3H1 on IκBα and A20 feedback 58
4.7. Influence of RC3H1 expression level on NF-κB signalling 61
4.7.1. Influence of RC3H1 expression levels on characteristic activation of NF-κB 63
4.7.2. Influence of RC3H1 expression levels on maximal concentration of NF-κB 64
4.7.3. Influence of RC3H1 expression levels on maximal concentration of IKK 67
4.7.4. Influence of RC3H1 expression level on the maximal concentration of NF-κB in the absence of the A20 feedback 69
4.7.5. Sensitivity analysis for the maximal concentration of NF-κB . 70
4.7.6. Influence of RC3H1 expression levels on the fold change of NF-κB 72
4.8. Discussion 74

5. Feedback modulation in cervical cancer cells 79
5.1. Motivation 79
5.2. Experimental Data 79
5.3. Mathematical Model 82
5.4. Stimulation strength influences NF-κB dynamics 84
5.5. Comparing dynamics of NF-κB in HeLa cells to HEK cells 85
5.6. Comparing kinetic parameter and concentrations of the canonical NF-κB pathway estimated for HeLa cells to HEK cells 87
5.7. Simulating post-transcriptional modulation of IκBα and A20 feedback with RC3H1 89
5.7.1. Influence of RC3H1 expression levels on maximal concentration of NF-κB 91
5.7.2. Sensitivity analysis for the maximal concentration of NF-κB . 93

5.7.3. Influence of RC3H1 expression levels on the fold change of NF-κB 94
5.8. Discussion 96

6. Conclusion and Outlook **99**

A. Modes of dynamical behaviour in mouse embryonic fibroblasts **103**
A.1. Mathematical model published by Kearns et al. [2006] 103
A.2. Mathematical model published by Ashall et al. [2009] 108
A.3. Core model derived from the model published by Kearns et al. [2006] 110
A.3.1. Modified core model 112

B. Feedback modulation in human embryonic kidney cells **113**
B.1. Supplemental Figures 113
B.2. Mathematical model of NF-κB activation in HEK cells 115

C. Feedback modulation in human cervical cancer cells **121**
C.1. Supplemental Figures 121
C.2. Mathematical model of NF-κB activation in HeLa cells 125

List of Figures **131**

List of Tables **135**

Abbreviations **137**

Bibliography **141**

Acknowledgements **153**