

Contents

List of Figures	13
List of Tables	17
List of abbreviations	19
1 Introduction	23
1.1 Quantitative dynamical modelling in synthetic biology	23
1.2 Contents of this thesis	23
1.2.1 Outline	23
1.2.2 Publications	23
2 Background	25
2.1 Introduction to synthetic biology	25
2.2 Introduction to Biochemical Modelling	26
2.2.1 Representation of transcription networks	26
2.2.1.1 Coupled chemical reactions and the law of mass action	26
2.2.1.2 Graphical representation of biochemical systems	27
2.2.1.3 Systems Biology Markup Language (SBML)	28
2.2.2 Modelling promoter regulation	28
2.2.2.1 Hill formalism	28
2.2.2.2 Shea-Ackers formalism	29
2.2.3 Simulation of dynamical systems	30
2.2.3.1 Ordinary differential equation (ODE)	30
2.2.3.2 Assumptions of deterministic modelling	31
2.2.4 Stochastic modelling of dynamical systems	32
2.2.4.1 Simulating stochastic models	32

2.2.4.2	The Gillespie algorithm	32
2.2.5	Phase plane analysis	32
2.2.6	Steady state and stability	32
2.3	Feedback loops and autoregulation	33
2.4	Parameter Inference	33
2.5	Biochemical system robustness	33
2.6	Introduction to Bayesian statistics	33
2.6.1	Bayes' theorem	34
2.6.2	Bayesian inference	34
2.6.3	Model checking	34
2.6.4	Prior selection	34
2.6.5	Model parametric Robustness	34
2.7	Approximate Bayesian Computation (ABC)	36
2.7.1	ABC algorithms	36
2.7.2	Particle sampling	38
2.7.3	Perturbation	38
2.7.4	Particle simulation	39
2.7.5	Weight calculation	39
2.8	The genetic toggle switch	41
2.8.1	Importance in natural systems	41
2.8.2	Uses in synthetic biology	42
2.8.3	Modelling the genetic toggle switch	42
2.9	Flow Cytometry	45
3	Postive feedback loops can increase the robustness of a genetic toggle switch	47
3.1	Introduction	47
3.2	Background	47
3.2.1	The bistable genetic toggle switch	49
3.2.2	Phase space and bifurcation analysis	49
3.3	Designing a simple synthetic switch	51
3.3.1	Parameter scan for model stability	51
3.3.2	Toggle switch parameter inference	56
3.3.3	Design specifications	57
3.3.3.1	Distance function	57
3.3.4	Results	58
3.4	Designing a more robust genetic toggle switch	60

3.4.1	Models of the genetic toggle switch	60
3.4.1.1	Autoregulation switches phase space and bifurcation analysis	62
3.4.2	ABC for model selection	65
3.5	Discussion	69
3.6	Summary	71
4	Dynamics of multi-stable switches	73
4.1	Introduction	73
4.2	Contributions to this Chapter	73
4.3	Background	73
4.4	Stability Finder algorithm	75
4.4.1	Algorithm overview	75
4.4.2	Initial condition sampling	77
4.4.3	Clustering methods	78
4.4.4	Distance function	78
4.4.5	Model checking	80
4.5	Calculating robustness	81
4.5.1	Case study 1: Infectious diseases	83
4.5.2	Case study 2: Population growth	84
4.6	Applications of Stability Finder	87
4.6.1	Testing StabilityFinder	87
4.6.2	Lu toggle switch models	91
4.6.2.1	Extending the Lu models	93
4.6.2.2	Multistability in the Lu models	97
4.6.2.3	Extending the Lu switch to three nodes	101
4.6.3	Mass action switches	103
4.6.3.1	Multistability in the MA switches	108
4.6.3.2	Robustness prior dependence	110
4.7	Discussion	114
4.8	Summary	115
5	Bayesian model fitting applied to flow cytometry data	117
5.1	Introduction	117
5.2	Contributions to this Chapter	117
5.3	Flow cytometry and model fitting	117
5.4	ABC-Flow algorithm development	118

10 CONTENTS

5.4.1	Intensity calculation	121
5.4.2	Distance Calculations	121
5.4.2.1	Kernel distance	122
5.4.2.2	Kolmogorov-Smirnov distance	126
5.4.2.3	Wald-Wolfowitz distance	127
5.5	ABC-Flow model fitting to simulated data	131
5.6	Toggle switch data collection	136
5.6.1	Circuit overview	136
5.6.2	Methods	138
5.6.2.1	<i>Escherichia coli</i> culturing conditions	138
5.6.2.2	Glycerol stock preparation	138
5.6.2.3	Revival	138
5.6.2.4	Plasmid construction	139
5.6.2.5	Polymerase Chain Reaction	139
5.6.2.6	Digestion	140
5.6.2.7	Agarose gel electrophoresis	140
5.6.2.8	Ligation	141
5.6.2.9	Transformation	141
5.6.2.10	Colony PCR	142
5.6.2.11	Sequencing	143
5.6.2.12	Inducers	143
5.6.2.13	Growth rate measurement	143
5.6.2.14	Flow cytometry	144
5.6.2.15	Concentration assays	144
5.6.2.16	Time course assays	145
5.6.3	Results	146
5.6.3.1	pKDL071 plasmid alteration	146
5.6.3.2	Control plasmids construction	146
5.6.3.3	Growth rate investigation	147
5.6.3.4	Toggle switch concentration assays	150
5.6.3.5	Toggle switch time course assay	154
5.7	ABC-Flow used on experimental data	157
5.7.1	Toggle switch model developed to fit to experimental data	157
5.7.2	Model fitting to the genetic toggle switch post ATc induction	160
5.7.3	Model fitting to the genetic toggle switch post IPTG induction	162
5.8	Discussion	165

5.9	Summary	167
6	Designing new switches	169
6.1	Introduction	169
6.2	Cloning overview	169
6.2.1	Resulting switches	171
6.3	Experimental design	171
6.3.1	Stage 1 - Construction of pKDL071-plac/ara-araC	171
6.3.2	Stage 2 - Construction of pKDL071-pluxtet-luxR	174
6.3.3	Stage 3 - Construction of pKDL0713a	176
6.4	Discussion	178
6.5	Summary	178
7	Conclusions	179
7.1	Evaluation	179
7.2	Future work	179
	Bibliography	181
	Appendices	189
A	Biochemical kinetic models	191
A.1	Ordinary differential equations	191
A.1.1	Standard toggle switch with inducers	191
A.1.2	Positive autoregulation on A and B with inducers	197
A.1.3	CS-MA	198
A.1.4	DP-MA	200
B	Primers	203
B.1	Primers used during PCR and sequencing	203
C	Algorithms	205
C.1	Clustering algorithms	205
C.1.1	Deterministic case	205
C.1.2	Stochastic case	205
C.2	K-means clustering	207
C.3	Two sample Kolmogorov-Smirnov test in 2D	207