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Master Thesis

Mathematical Models of  
Adipogenesis



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# Abstract

## English

Obesity is nowadays one of the most severe health problems. It causes different diseases that reduce life quality. The existence of a mathematical model that could simplify the testing of new research ideas, before time and costs consuming *in vitro* and *in vivo* experiments are employed, is of major interest and importance.

Here three different models of adipogenesis are presented. One model consists of a system of ordinary differential equations and is able to reproduce the gene expression profiles of some of the well known adipogenesis regulators, inhibitors and markers like Ppar $\gamma$ , Cebp $\alpha$ , Gilz, Gata2, Fabp4 and Scd1. Another model is based on an echo state network that models the interactions between the key players of adipogenesis. This model is capable of predicting the gene expression levels in response to a certain adipogenic cocktail out of the gene expression levels in response to other adipogenic cocktails. The third model is based on so called "essential genes". From these genes a subset is chosen to be employed in the parameter estimation of the model. The "impact" of each gene on the dynamic behavior of the model is computed. In this way a list of new genes, sustained by biological research, is proposed for further studies. These genes could play an important role in the process of adipogenesis.

**Keywords:** adipogenesis, mathematical model, *in silico*, differential equation, neural network, "essential" genes

## German

Übergewicht ist ein Gesundheitsproblem welches immer mehr Menschen betrifft. Es verursacht Krankheiten die die Lebensqualität einschränkt. Um auf diesem Gebiet schneller und effektiver Fortschritte zu erzielen würde ein mathematisches Modell für die Adipogenese vom großen Nutzen sein.

Es werden drei Modelle erarbeitet. Das erste wird durch ein System von Differentialgleichungen dargestellt. Dieses ist in der Lage die Expressionsprofile der wichtigsten Adipogeneseregulatoren, -Inhibitoren und -Marker wie Ppar $\gamma$ , Cebp $\alpha$ , Gilz, Gata2, Fabp4 and Scd1 vorherzusagen. Ein weiteres Modell beschreibt die Interaktionen zwischen den bekanntesten Adipogenesefaktoren durch ein Echo State Netzwerk. So ist es möglich die Expressionsprofile der Gene, die von einem bestimmten Adipogenesecocktail verursacht werden, zu berechnen. Für das dritte Modell wird zuerst eine Liste von so genannten "essentiellen Genen" erstellt. Aus dieser Liste werden dann Gene bestimmt die in das Modell eingesetzt werden. Der "Einfluss" dieser Gene wird berechnet. Somit werden neue Gene vorgeschlagen die eine wichtige Rolle in der Adipogenese spielen könnten.

**Stichwörter: Adipogenese, mathematisches Modell, *in silico*, Differentialgleichung, Echo State Netzwerk, "essentielle" Gene**

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# Chapter 1

## Introduction

Nowadays obesity constitutes a severe health problem. Affected people tend to develop cardiovascular diseases and NIDDM (non-insulin-dependent diabetes mellitus). Formation of adipose tissue is due to two processes. New fat cells develop and the total fat cell number increases (hyperplasia). The already existing fat cells increase their storage capacity of triglycerides (hypertrophy). A detailed understanding of the mechanisms governing these processes would be an important step in preventing obesity and the related health problems.

*In vitro* the process can be studied using different cell lines. The most often used are the 3T3-L1 and 3T3-FA442A lines originally created by Green and Kehinde [11, 12]. Numerous *in vitro* and *in vivo* experiments have discovered some of the transcription factors, adipogenic factors and cell-cycle factors that play a key role during adipogenesis.

The development of preadipocytes into adipocytes is called adipogenesis. For a detailed description see [9]. *In vitro* the process is induced by exposure of the preadipocytes to a cocktail consisting of fetal bovine serum (FBS), dexamethasone (DEX), isobuthymethylxanthine (IBMX) and insulin. As a result, the cells enter the clonal expansion phase followed by terminal differentiation. During the clonal expansion of 3T3-L1 cells at least one cell-cycle is traversed. The terminal differentiation phase is marked by metabolic programs typical for mature fat cells.

A detailed review of the existing information on the key players required for a successful adipogenesis can be found in [31, 8]. The master regulators are considered to be Pparg and Cebpa. Evidence [26] shows that Pparg is capable of launching the process of adipogenesis; Pparg is necessary and sufficient. In [7] it is suggested that Cebpa induces adipogenesis through Pparg. There are evidence that the cross-regulation of Cebpa and Pparg controls the transcriptional pathway of adipogenesis [36]. Two other important regula-

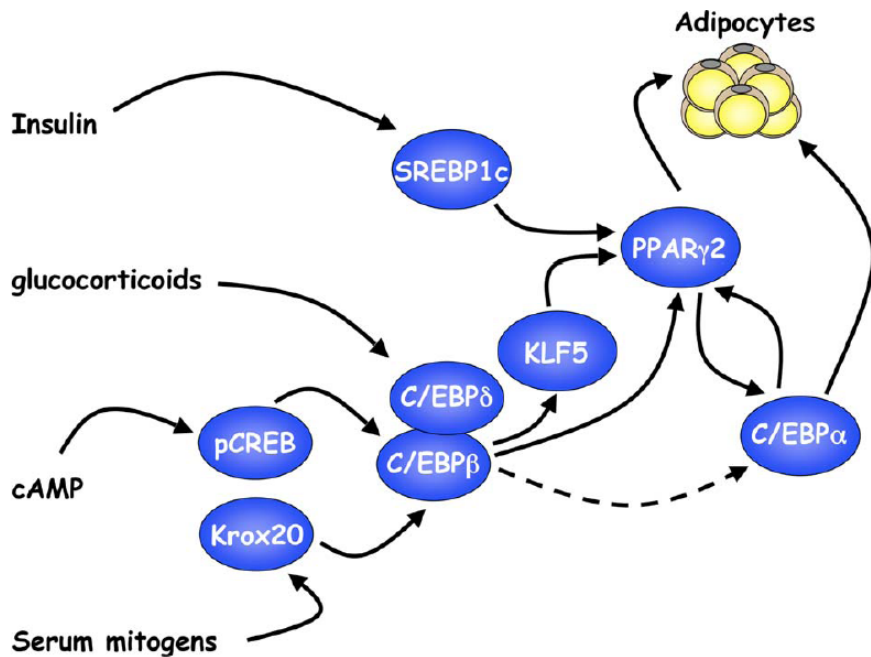


Figure 1.1: Positive transcription factors (adopted from [31])

tors are Cebpb and Cebpδ. They induce the expression of Pparg and Cebpa. Other adipogenic factors are SREBP1c, KLF5, pCREB, Krox20 and cAMP. An overview of the interplay of the mentioned factors is presented in Figure 1.1. Other regulators are cell-cycle-related proteins and some negative factors that inhibit the expression of Pparg, Cebpa, Cebpb and Cebpδ. In [31] the negative factors and some of the most studied positive factors that control adipogenesis are summarized into one diagram (see Figure 1.2).

In order to discover the factors that determine a preadipocyte to develop into an adipocyte, high-throughput analysis methods and proteomics can be used. One of the most efficient way to measure gene expression is microarray analysis. It was developed in the 1990s and today is one of the most often used analysis technique. One reason that led to this is the possibility of measuring thousands of genes simultaneously. Proteomics stands for large-scale studies of protein structure and functions. This analysis method is more complex because of the diversity of possible structures and functions. Analysis of individual proteins is more simple. The Western blot technique is employed when protein levels have to be measured.

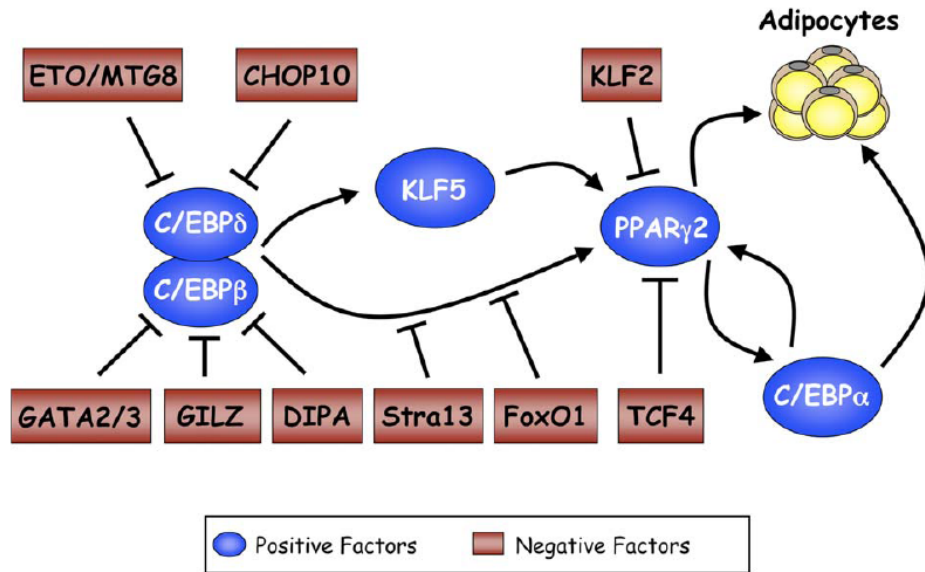


Figure 1.2: Positive and negative regulators of adipogenesis (adopted from [31])

## 1.1 Objectives

Although until now a lot of experiments were conducted in order to elucidate the factors and their interactions responsible for the development of preadipocytes into adipocytes, no mathematical models of the biological process are available. A model would enable *in silico* testing of different hypotheses without the costs and time needed for *in vitro* and *in vivo* testing.

Starting from the available information on adipogenesis, the key factors involved and a number of large scale experiments, three mathematical models were derived.

- One model combined the factors in Figure 1.1 and Figure 1.2. The regulation was described by a system of ordinary differential equations [5] whose coefficients were determined from measured data.
- One model used an echo state network for the interaction of the factors. The input to the echo state network was the time course of the adipogenic cocktail. The output of the model was the time course of two proteins known as markers for adipocytes.
- One model was based on "essential" genes. The relationship between them was specified by differential equations. The parameters of the model were determined from measured data.

# Chapter 2

## Methods

In the first section of this chapter the concept of the differential equation model is described. The second section shows how an echo state network was used to model the process of adipogenesis. In the last section of this chapter the third model is presented.

### 2.1 Ordinary differential equation model

Many of the factors involved in the process of adipogenesis are known. Some of them are considered to play a key role because their absence would lead to a stop in the development of preadipocytes into adipocytes. Current knowledge is gathered in the review article [31] and presented in Figure 1.1 and Figure 1.2. For this model the shown factors are extended by two adipocyte markers. Figure 2.1 presents these factors and their dependencies.

#### 2.1.1 Gene expression data

Raw data (.cel files) from an adipocyte differentiation microarray experiment [1, 18] were downloaded from Gene Expression Omnibus (GEO) (GSE6794). 3T3-L1 fibroblasts were cultured in vitro and induced to differentiate using standard MDI protocol. At successive time-points (PC, 0h, 6h, 12h, 24h, 48h, 3d, 4d, 7d, 28d) cells were collected, and processed for microarray analysis using Affymetrix Murine 11k A and B arrays. Data were normalized using `gcrma` (R/Bioconductor) and results from the Mu11kA array were combined with results for complementary probesets of Mu11kB arrays. Relative gene expression levels ( $\log_2$ ratios) from each time point versus the pre-confluent state were determined and averaged over 3 biological replicates.

Since between gene expression levels on day 7 and day 28 of differentiation

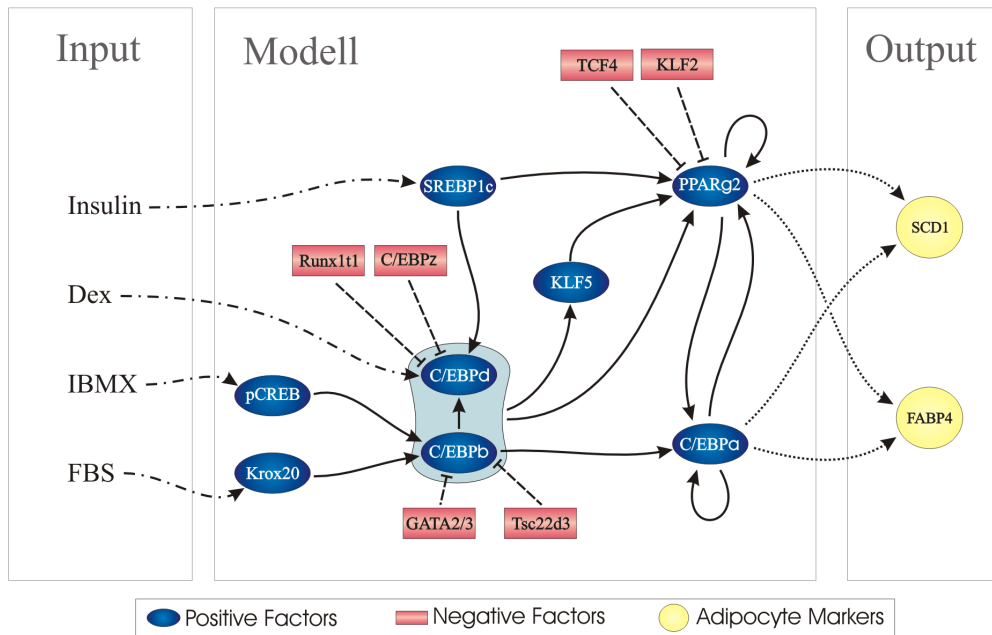


Figure 2.1: Interplay of the key regulators of adipogenesis and adipocyte markers. The regulators are divided into positive factors (blue ellipses) and negative factors (pink rectangulars). The yellow circles stand for the adipocyte markers. The influence of the input cocktail is marked by chain dotted arrows. Positive regulation is denoted by regular arrows and the negative regulation by dashed arrows. The markers are determined by the master regulators *Cebpa* and *Pparg* (dotted arrows).

no major differences exist, the last time point of the measurements was not considered. In order to obtain a continuous signal representing the change in the expression of each gene, the measured data was interpolated using cubic splines with a resolution of one minute. The data processed in this way was used in the differential equation model.

## 2.1.2 Model description

The model was described by system of ordinary differential equations [5, 4]. Each factor that is known to exhibit positive regulation was described through an ordinary differential equation (ODE). The factors that are responsible for negative regulation were present in the ODEs of the other factors, but were not be described by an ODE. This was due to the fact that, because of simplicity reasons, these factors had no input.

Looking at Figure 2.1, the ODE of *Cebpa* was written as:

$$\frac{d Cebpa(t)}{dt} = a_1 * Pparg(t) + a_2 * Cebpb(t) + a_3 * Cebpa(t)$$

*Cebpa(t)*, *Cebpb(t)*, *Pparg(t)* were the gene expression levels of *Cebpa*, *Cebpb* and *Pparg* considered as functions of time. In this way each of the regulators was described.  $a_1$ ,  $a_2$ ,  $a_3$  were the parameters that scale the influence of each of the involved genes. The system consisting of 10 ODEs and 28 parameters is shown below. The parameters were determined based on microarray data.

$$\left\{ \begin{array}{l} \frac{d Cebpa(t)}{dt} = a_1 * Pparg(t) + a_2 * Cebpb(t) + a_3 * Cebpa(t) \\ \frac{d Pparg(t)}{dt} = a_4 * Srebp1c(t) + a_5 * Klf5(t) + a_6 * Cebpa(t) \\ \quad + a_7 * (Cebpb(t) + Cebpd(t)) + a_8 * Pparg(t) \\ \quad + a_9 * Klf2(t) + a_{10} * Tcf4(t) \\ \frac{d Cebpb(t)}{dt} = a_{11} * Creb(t) + a_{12} * Krox20(t) + a_{13} * Gata23(t) \\ \quad + a_{14} * Tsc22d3(t) \\ \frac{d Cebpd(t)}{dt} = a_{15} * DEX(t) + a_{16} * Srebp1c(t) + a_{17} * Cebpb(t) \\ \quad + a_{18} * Runx1t1(1) + a_{19} * Cebp\zeta \\ \frac{d Srebp1c(t)}{dt} = a_{20} * Insulin \\ \frac{d Klf5(t)}{dt} = a_{21} * Cebpb(t) + a_{22} * Cebpd \\ \frac{d Krox20(t)}{dt} = a_{23} * FBS(t) \\ \frac{d Creb(t)}{dt} = a_{24} * IBMX(t) \\ \frac{d Fabp4(t)}{dt} = a_{25} * Pparg(t) + a_{26} * Cebpa(t) \\ \frac{d Scd1(t)}{dt} = a_{27} * Pparg(t) + a_{28} * Cebpa(t) \end{array} \right.$$

The input to the system consisted of the time course of the adipogenic cocktail (see Figure 2.1.2). The values of each substance in the cocktail can not be compared to its real concentration in *in vitro* experiments. The

values used in the model proved to be useful during parameter estimation. The time courses of the gene expression of *Fabp4* and *Scd1* constituted the output of the model. In this case these two proteins were considered markers for mature adipocytes.

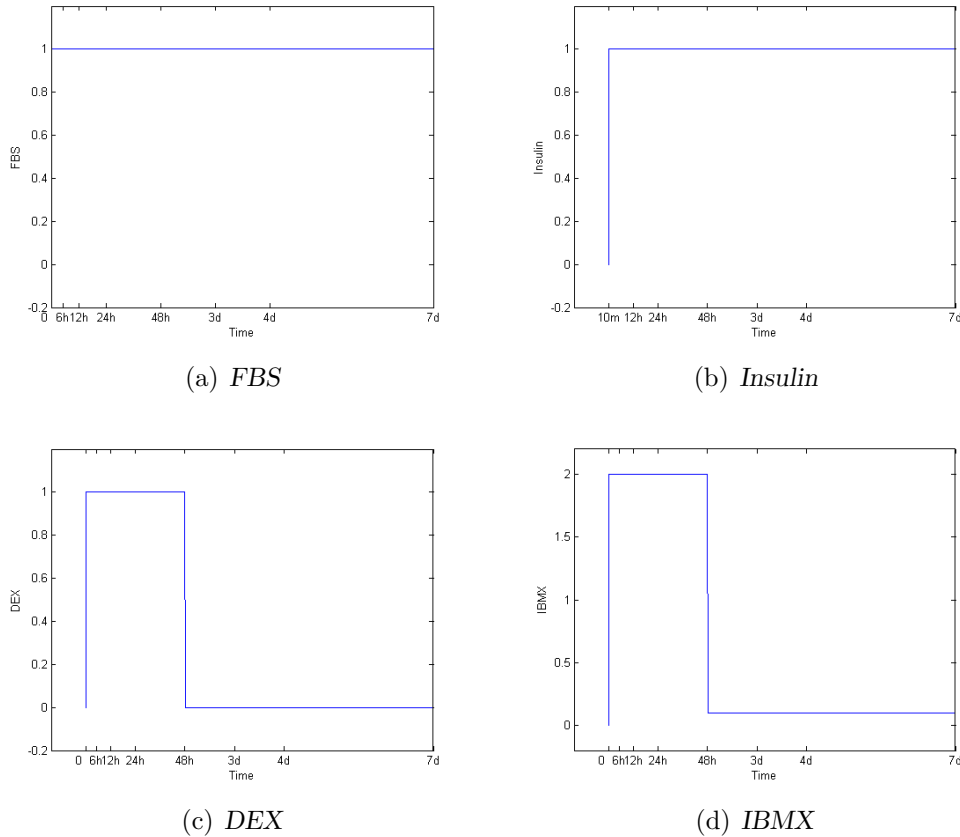


Figure 2.2: Standard adipogenic cocktail

### 2.1.3 Implementation

The parameters of the model were determined so that the gene expression level of the proteins matched the measured data. This task was done using the SBToolbox2. This is a toolbox for systems biology that was developed for Matlab (Mathworks Inc., Natick, USA).

The SBToolbox2 [14] provides functionality for modeling, simulation and analysis of biochemical systems. This toolbox requires Matlab version 7.1 or higher and the SBPD project for the parameter estimation functionality.

The system of ODEs was transferred to Matlab and the parameter estimation tool was used. Since for the estimation of the 29 parameters only one microarray experiment was available, the estimation was poor. The computed values were used as a starting point. The system was simulated with these values and the resulted expression levels of the genes were compared to the measurements. The SBToolbox2 allows the use of events. Events define changes in the parameters or ODEs of the system. With the help of events, the values of the parameters were changed at certain time points during simulation so that the measured data could be reproduced by the model.

## 2.2 Echo state network model

In the previous section, the interaction between the regulators of adipogenesis was modeled using ordinary differential equations. In this section the interactions were modeled using an echo state network [16].

### 2.2.1 Principles of echo state networks

Neural networks [24, 35] can be divided into two types. There are feedforward networks and recurrent networks. The typical structure of these two types of networks is presented in Figure 2.3. As shown, the major difference is the "direction" of information processing. In the feedforward neural network the activation travels from the input through the hidden units (if present) and then reaches the output. In the recurrent neural network there is at least on cyclic path that the activation passes through. An echo state network is a special recurrent neural network in which only certain connections are trained while others remain fix.

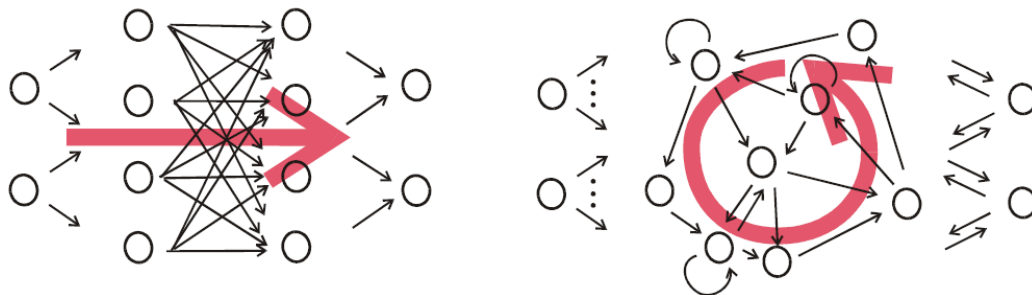


Figure 2.3: Typical structure of a feedforward network and a recurrent network (adopted from [16]). The direction of the information processing is marked with red arrows.

	DMI	DM	DI	MI	D	M	I
Cebpa	100%	130%	0%	0%	0%	0%	0%
Fabp4	100%	80%	90%	0%	90%	0%	0%

Table 2.1: Protein levels in response to different input cocktails (derived from 2.4)

## 2.2.2 Gene expression and protein level data

For this model the same gene expression data (see subsection 2.1.1) as for the ODE model was used, as well as a protein levels of differentiating 3T3-L1 preadipocytes that were presented in [15]. The measurements of the protein levels were done on day five of adipogenesis. The results are shown in Figure 2.4. This experiment was used in an unconventional way.

The protein levels in response to DMI was considered to be 100%. In addition, this protein levels were considered to be equivalent to the corresponding gene expression measured on day five of the microarray experiment. The protein levels measured in response to the other adipogenic cocktails (Table 3.1) were determined as percentage of the response to DMI. Table 2.1 was derived from Figure 2.4. 0% percent was used when the protein levels in response to the adipogenic cocktails were equal to the NCS case. The measured gene expression levels in response to DMI were multiplied with the percentage values from Table 2.1 to obtain the corresponding gene expression levels in response to the other adipogenic cocktails.

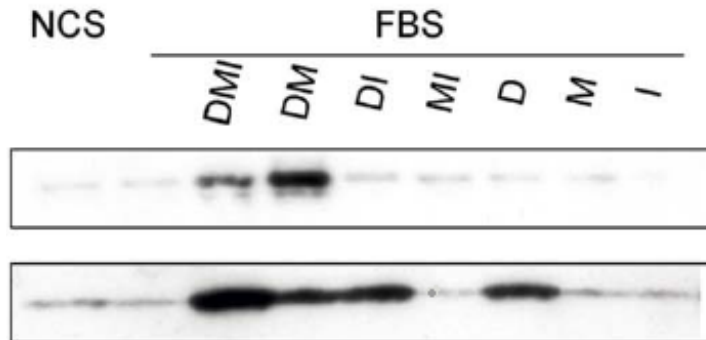


Figure 2.4: Protein levels of Cebpa (upper part) and Fabp4 (lower part) determined using western blots (adopted from [15])

### 2.2.3 Model description

The echo state network (ESN) used in this approach was shown in Figure 2.5. The choice of an ESN for this task was based on the fact that all biological networks are recurrent networks.

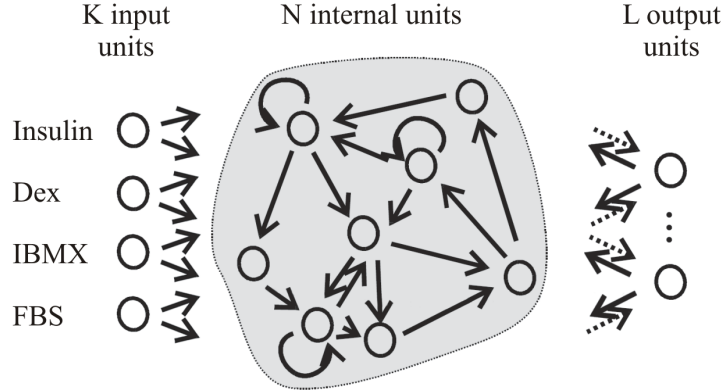


Figure 2.5: Structure of the echo state network modeling the differentiation process (adopted from [16]). The dotted arrows stand for connections that are trained. All other connections are fixed. The internal units (on the gray background) form the **dynamic reservoir** of the echo state network.

The most important mathematical characteristics (see [16]) of the ESN model are summarized here. The input to the ESN network was referred to as  $\mathbf{u}(n)$ . The vector describing the state of the internal units was called  $\mathbf{x}(n)$ .  $\mathbf{y}(n)$  was the output of the network.  $n$  denoted the time points of the simulation. The ESN model possessed  $K$  input units,  $N$  internal units and  $L$  output units. The input was connected to the dynamic reservoir through the matrix of weights  $\mathbf{W}^{in} \in \mathbb{R}^{N \times K}$ . The weights of the dynamic reservoir were gathered in the matrix  $\mathbf{W} \in \mathbb{R}^{N \times N}$ . The matrix  $\mathbf{W}^{out} \in \mathbb{R}^{L \times (K+N)}$  contained the weights from the dynamic reservoir to the output units. Since there also existed connections from the output units to the dynamic reservoir there was also the matrix of these weights  $\mathbf{W}^{back} \in \mathbb{R}^{N \times L}$ .

The activation of the internal units was computed using the following equation:

$$\mathbf{x}(n+1) = \mathbf{f}(W^{in}\mathbf{u}(n+1) + \mathbf{W}\mathbf{u}(n) + \mathbf{W}^{back}\mathbf{y}(n)), \quad (2.1)$$

where  $\mathbf{f}$  was the component wise transfer function of the internal units,  $\mathbf{u}(n+1)$  was the external input. The output of the ESN was computed with the following equation

$$\mathbf{y}(n+1) = \mathbf{f}^{out}(\mathbf{W}^{out}(\mathbf{u}(n+1), \mathbf{x}(n+1), \mathbf{y}(n))), \quad (2.2)$$

where  $(\mathbf{u}(n+1), \mathbf{x}(n+1), \mathbf{y}(n))$  denoted a vector resulted from the concatenation of the input vector, internal state vector and output vector.  $\mathbf{f}^{out}$  was the transfer function of the output units.

The Model had four input units, each one receiving the time course of an ingredient of the adipogenic cocktail. Through testing, the following parameters of the ESN were the best choice.

- the dynamic reservoir had 30 units
- 20% of the reservoir units received the input signals
- the inputs were scaled with 0.9
- the transfer function of the reservoir units was tanh
- 10% of the reservoir received feedback from the output
- the feedback from the output was scaled with 0.1
- the output units used a linear transfer function
- the output weights were learned using Bayesian regularization in a Levenberg-Marquardt algorithm

## 2.2.4 Implementation

The ESN model was implemented in Matlab. The mechanisms of the ESN were implemented in concordance with the formulas presented earlier. The Bayesian regularization method used for the computation of the output weights was provided by Matlab. When leave-one-out cross validation was used, the training presented some subtleties. Since six different input/output experiments were used for training, it was important that between the different experiments, the ESN had enough time to prepare for the next experiment. The solution was to feed the network with noise between the different experiments.

In order to test the model, two scenarios were created. In the first scenario the ESN model was simulated using DMI as input cocktail. In this case the time course of the genes expression levels of the adipogenesis regulators constituted the output of the network (16 output units). The same regulators as in the ODE model were used. The ESN model was tested on the data derived from the western blots.

In the second scenario, the ESN was used in a slightly different manner. The concept of leave-one-out cross validation was applied. For this, the

I/O cases	Train	Test
Case I	DMI, DM, DI, MI, D, M	I
Case M	DMI, DM, DI, MI, D, I	M
Case D	DMI, DM, DI, MI, M, I	D
Case MI	DMI, DM, DI, D, M, I	MI
Case DI	DMI, DM, MI, D, M, I	DI
Case DM	DMI, MI, DI, D, M, I	DM
Case DMI	DM, MI, DI, D, M, I	DMI

*Table 2.2: ESN model - input/output cases. Here the exact train and test data is presented. The name of the cases corresponds to the adipogenic cocktail that was used for testing.*

microarray analysis data and the wester blot data was needed. Out of the measured protein levels, proportional gene expression levels were computed. In this way seven different input/output cases were available. These were used in the following way. Six of them were employed in the training phase and the seventh was used in the testing phase. This was done for each of the resulted input/output cases during seven simulations of the model. During each simulation a new set of parameters, that corresponded to a new ESN model, were computed. The exact train and test data are presented in Table 2.2. The ESN model had 2 output units because during western blotting only Cebpa and Fabp4 were measured. The Matlab code for this scenario is provided in the Appendix.

## 2.3 "Essential" genes model

In the previous two sections, the process of adipogenesis was modeled using two different models. In the beginning, a system of ordinary differential equations was used. Afterwards an echo state network modeled the interactions between the regulators of the process. In both approaches, the used regulators are well known key players of adipogenesis (see [31] and [8]). In this third way of describing adipogenesis the well known regulators were not be employed anymore. First, a set of "essential" genes was determined. Then, these genes were used to model adipogenesis. For this model the name essential genes model (EG model) will be used.

### 2.3.1 "Essential" genes

Out of different experiments a set of new genes with common characteristics were identified. In [15] a list of IBMX regulated genes was determined. For this, 3T3L1 cells were treated with DMI or DI. Out of this data, the IBMX regulated genes were identified. A list of DEX-regulated genes is presented in [6]. In order to identify (see Figure 2.6) the "essential" genes, first a list of IBMX and DEX regulated genes was set up. The genes contained in this list were then compared to results from three different microarray experiments. A gene is considered to be an essential gene if in at least one of these experiments a log fold change of at least one is measured.

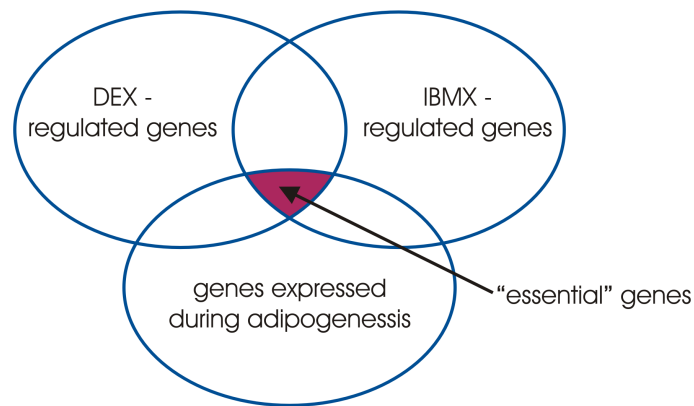


Figure 2.6: Selection of the "essential" genes.

The interactions between the identified genes were determined using STRING 8. In this way, a network view was generated. The interactions between the genes were computed by STRING 8 from different sources including experimental repositories, computational prediction methods and public text collections. Based on the computed interactions a subset of genes was selected to be used in the modeling process. This subset consisted of genes that presented at least four connections to the other genes. The network view was generated using Cytoscape [25]. In Figure 3.11 not only the "essential genes" and their connections can be seen but also the genes selected for modeling. Cytoscape is an open source software project allowing, among other things, the integration of biomolecular interaction networks with expression data in a unified framework.

The time courses of the gene expression levels were computed by cubic interpolation. The used data included the microarray data used in the ODE and ESN model [1] and results from two other microarray experiments.

3T3L1 and MEF cells were measured during induced adipogenesis. The results from the 3T3-L1 [13] and MEF cells are available under ArrayExpress E-MARS-2 and ArrayExpress E-MARS-13.

The heat map of the gene expression levels was plotted using Genesis [3, 2] and can be seen in Figure 3.6. Some of the expression profiles shown resulted from the microarray experiment done by Soukas et al. and some of the profiles were interpolated from microarray experiments done by Hackl et al.. In this way a unified heat map of the essential genes was generated.

### 2.3.2 Model description

Inspired by the model described in [10, 29] for keratinocyte migration, a similar model for adipogenesis was formulated. The "essential" genes were employed in a neural network model. The dynamics of the model were defined as a system of differential equations. Such a differential equation is presented by equation 2.3.

$$\frac{dg_i(t)}{dt} = \frac{1}{\tau_i} \left( g_i(t-1) + \sum_{j=1}^N w_{ij} * A(g_j(t-1) - \delta_j) \right) + I_i * \exp(k_i * t) \quad (2.3)$$

The functions and variables used in 2.3 had different meanings. The change in the gene expression level of gene  $g_i$  depended on the other  $N$  genes. The time constant of the change was  $\tau_i$ . The activation function of the genes was called  $A$  and was defined as  $A(x) = \frac{1}{1+\exp(-x)}$ .  $\delta_j$  was an offset term. The term  $I_i * \exp(k_i * t)$  accounted for the external input to each gene. The weights  $w_{ij}$  described the strength of the interaction between gene  $g_i$  and gene  $g_j$ .

For each of the  $N$  genes  $g_i$  the following parameter were determined:

- 1 time constant  $\tau_i$
- $N$  weights  $w_{ij}$  connecting gene  $g_i$  to the other  $N$  genes
- 1 offset term  $\delta_i$
- 1 amplitude  $I_i$  of external input
- 1 argument  $k_i$  of the external input

For this model a total number of  $N*(N+4)$  parameters were determined.

### 2.3.3 Implementation

The identification of the "essential" genes was implemented using the programming language C. The parameters of the model were determined using Matlab and the provided Genetic Algorithm Toolbox. The parameters were determined so that the mean squared error between the measurements and the simulation was minimized. The mean squared error (MSE) between the measurements  $M$  and the simulation  $S$  for  $N$  genes and  $T$  time points was defined as:

$$MSE = \frac{1}{TN} \sum_{i=1}^N \sum_{t=1}^T (M(i, t) - S(i, t))^2 \quad (2.4)$$

The parameters of the model were determined by a genetic algorithm [22, 23, 27] employing 5000 generations, each with a population of  $N*(N+4)*10$  individuals.

# Chapter 3

## Results

In the first subsection the results of the ODE model are shown. The results of the second model follow in the second subsection. The third subsection is dedicated to the results obtained from the third model. These results are discussed in the next chapter.

### 3.1 ODE model can compute gene expression profiles of employed regulators

The model described in subsection 2.1.2 on page 7 was simulated using the SBToolbox2 ([14]). The computed time courses of the employed regulators matched the measured data. Gene expression levels of the two adipocyte markers (Figure 3.1) were shown. The gene expression levels of Pparg and Cebpa (Figure 6.1) which are considered to be the master regulators of adipogenesis were presented in the Appendix. These results were obtained if the model's input was the well-known adipogenic cocktail (Insulin, DEX, IBMX and FBS). The time courses of the other regulators exhibited the same similarity to the measured data.

The model was also simulated for other different inputs. Three simulations were performed in which either Insulin, DEX or IBMX was not used. In other three simulations FBS together with either Insulin, DEX or IBMX formed the input. These six different input cocktails together with the initial input cocktail were described in Table 3.1. The ingredients of each cocktail were marked by an "x" in their corresponding table box.

The simulation results for the different input cocktails were shown in Figure 3.1 and Figure 6.1. Figure 6.1 can be found in the Appendix. Only the gene expression levels of Cebpa were plotted. In [15] the protein levels of Cebpa for the different input cocktails were determined using western blots.

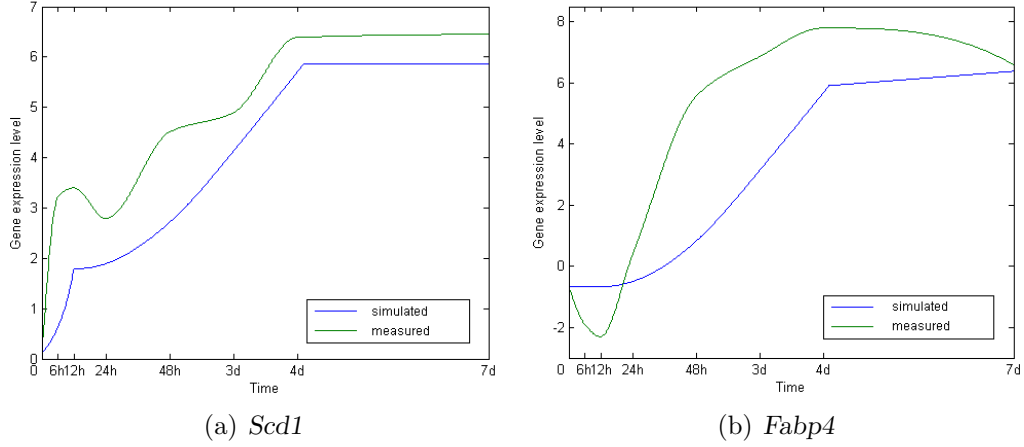


Figure 3.1: ODM model: gene expression levels of adipocyte markers

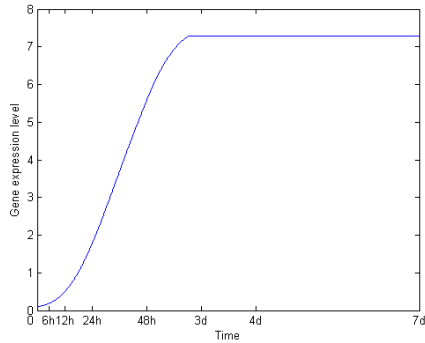
Input	FBS	Insulin	DEX	IBMX
DMI	x	x	x	x
DM	x		x	x
DI	x	x	x	
MI	x	x		x
D	x		x	
M	x			x
I	x	x		

Table 3.1: Ingredients of the different input cocktails

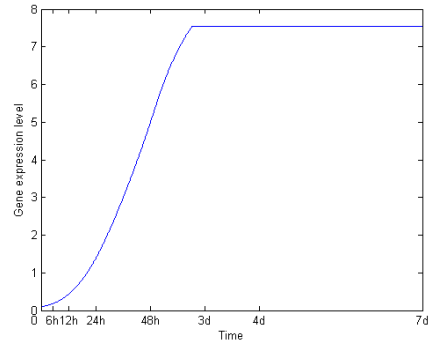
### 3.2 ESN model predicts higher gene expression of Cebpa in the absence of Insulin

The ESN model described in section 2.2 on page 10 was employed in two scenarios. First the model was trained using DMI as input cocktail. The output units computed the time courses of the gene expression levels of the regulators of adipogenesis. The gene expression levels simulated during the training phase, together with the measured gene expression levels, were shown in Figure 3.2. Here only the gene expression levels for two genes were shown. The quality of the simulated gene expression levels of the other genes was similar.

Then the network was tested using different adipogenic cocktails (Table 3.1). Figure 3.4 showed the simulated gene expression levels for the case in which DM was used as input cocktail.

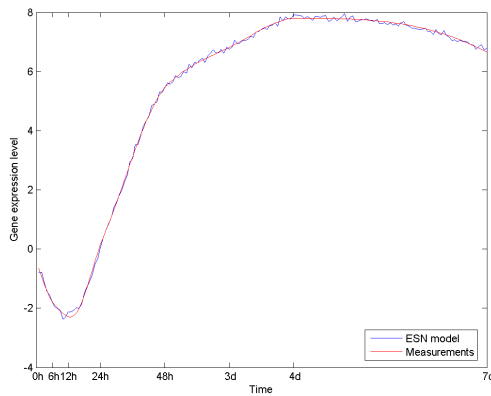


(a) *Input cocktail DM*

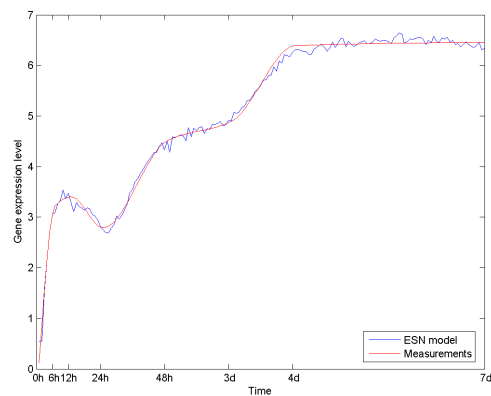


(b) *Input cocktail DI*

Figure 3.2: ODM model: gene expression level of *Cebpa* for different input cocktails



(a) *Fabp4*



(b) *Scd1*

Figure 3.3: ESN model: simulated and measured gene expression levels of *Fabp4* and *Scd1* during the training phase

### 3.3 ESN model is able to predict correct gene expression levels of *Cebpa* and *Fabp4* in response to Insulin

In the second scenario the concept of leave-one-out cross validation was used. The results in response to I were shown in Figure 3.5. The results in response to DMI and D were presented in Figure 6.3 and 6.4 in the Appendix.

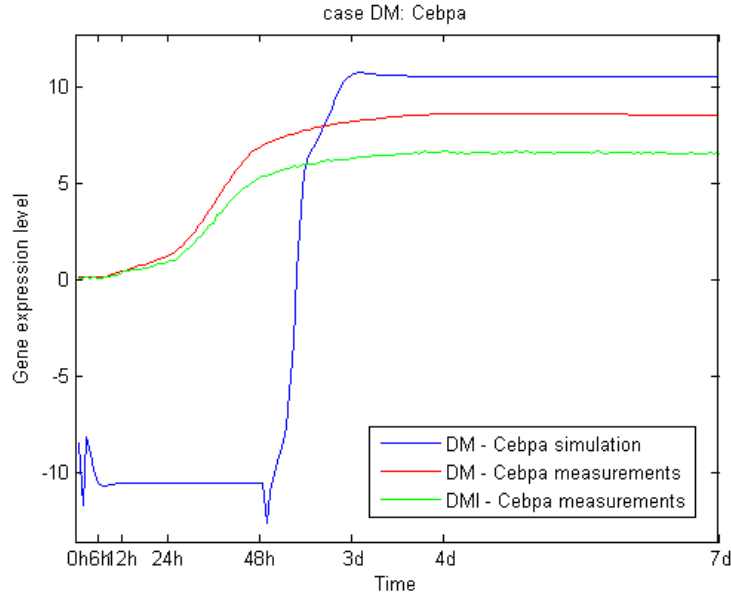


Figure 3.4: ESN model - case DM: Time courses of Cebpa

	Bdnf	Bhlhb	Cd36	Cebpa	Egr1	Id2	Myc	Pparg
$\tau$	-2,611	-1,668	-12,67	-4,410	-0,791	-1,384	-1,151	-3,316
$\delta$	1,554	1,373	1,664	1,916	0,037	-1,588	1,261	2,132
$I$	1,229	0,556	0,104	0,507	1,033	0,438	1,851	0,701
$k$	-0,035	-0,011	-0,005	0,001	0,003	0,006	-0,001	-0,003

Table 3.2: EG model - values of the parameters  $\tau$ ,  $\delta$ ,  $I$  and  $k$ .

### 3.4 EG model proposes a list of new genes that could play important roles during adipogenesis

This section is dedicated to the results obtained by the EG model described in section 2.3 on page 14. First a set of "essential" genes was determined. These genes were shown in Figure 3.11. The heat map of the genes was presented in 3.6. Out of these genes a subset was chosen for modeling. The selected genes were shown in red circles in Figure 3.11.

These selected genes were connected by parameters in a system of differential equations. The heat map of the weights was shown in Figure 3.7. The other parameters were presented in Table 3.2.

Based on the computed weights of the EG model a diagram of the network

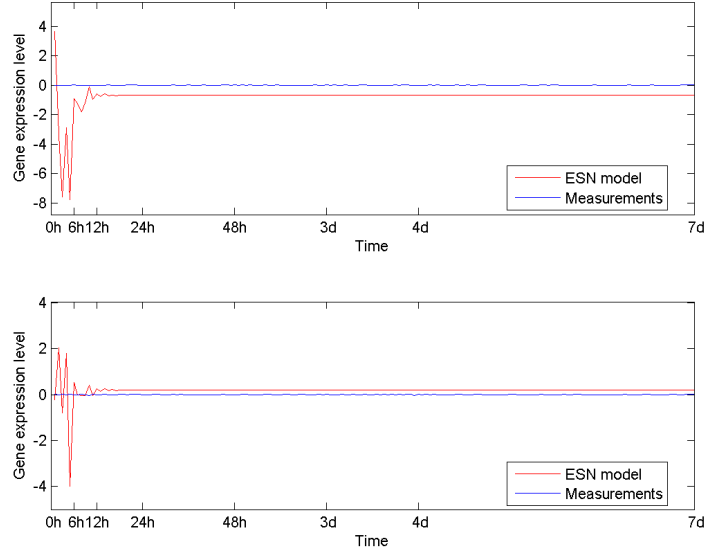


Figure 3.5: ESN model - case I using leave-one-out cross validation: gene expression level of *Cebpa* (upper plot) and *Fabp4* (lower plot) during testing phase

was drawn. The weights having there an absolute value higher than four were employed. The strength of the drawn connection was proportional to the values of the weights. The sign of the weight values was shown using different types of arrows. The resulted network can be seen in Figure 3.8.

The parameters were computed so that the mean square error between the simulated and measured gene expression levels was minimized. With the determined parameters, the mean squared error is equal to 0.15. The simulated and measured gene expression profiles are presented in Figure 3.9.

After the parameters of the model were determined, the "impact" of each gene on the dynamic behavior of the model was determined. The impact was measured as the mean squared change in the gene expression levels. The results are shown in Figure 3.10.

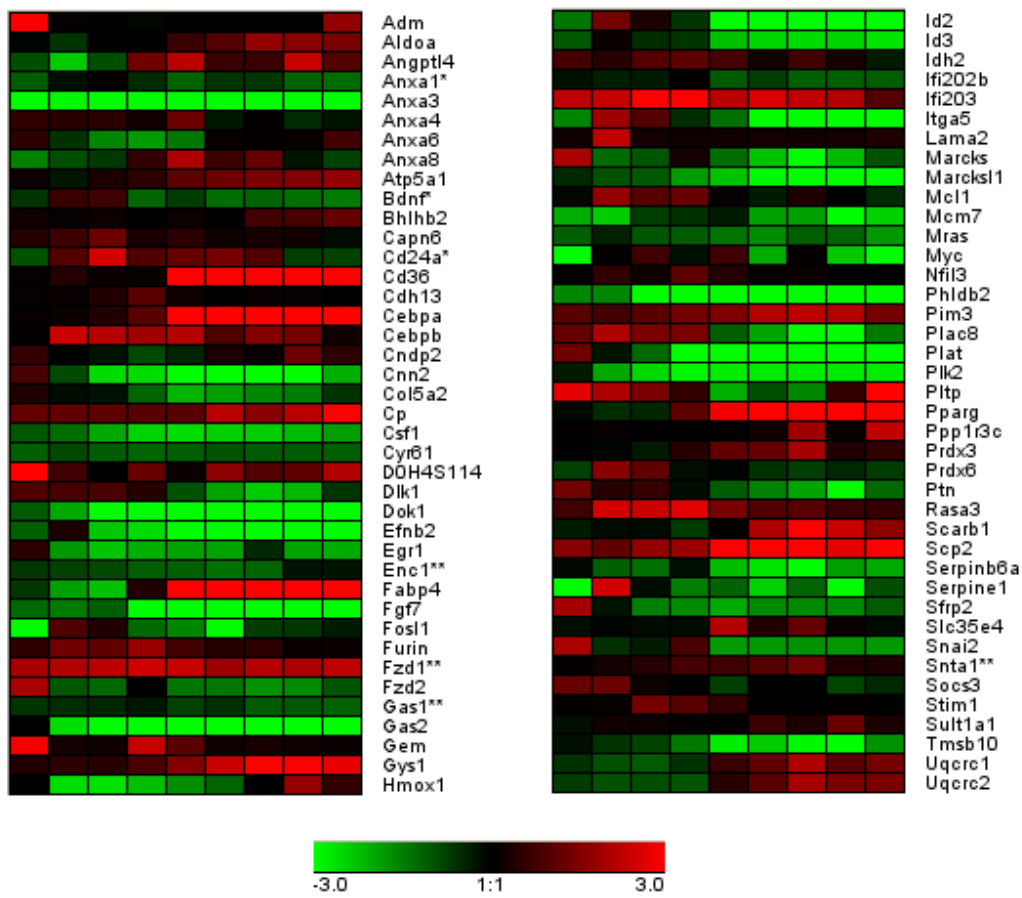


Figure 3.6: Essential genes model - heat map of the 80 identified essential genes.

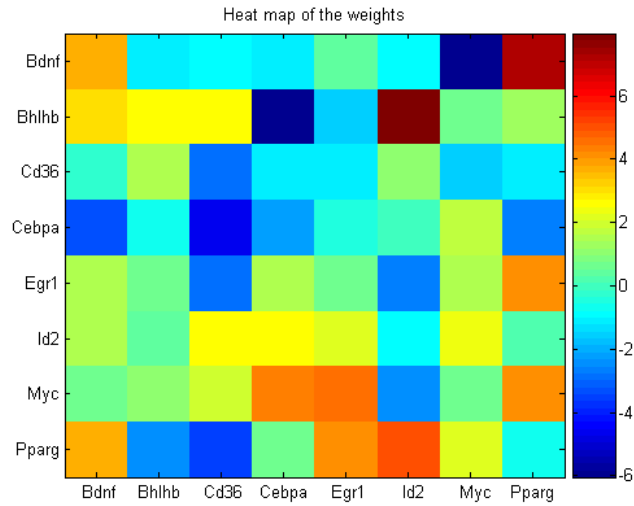


Figure 3.7: EG model - heat map of the estimated weights

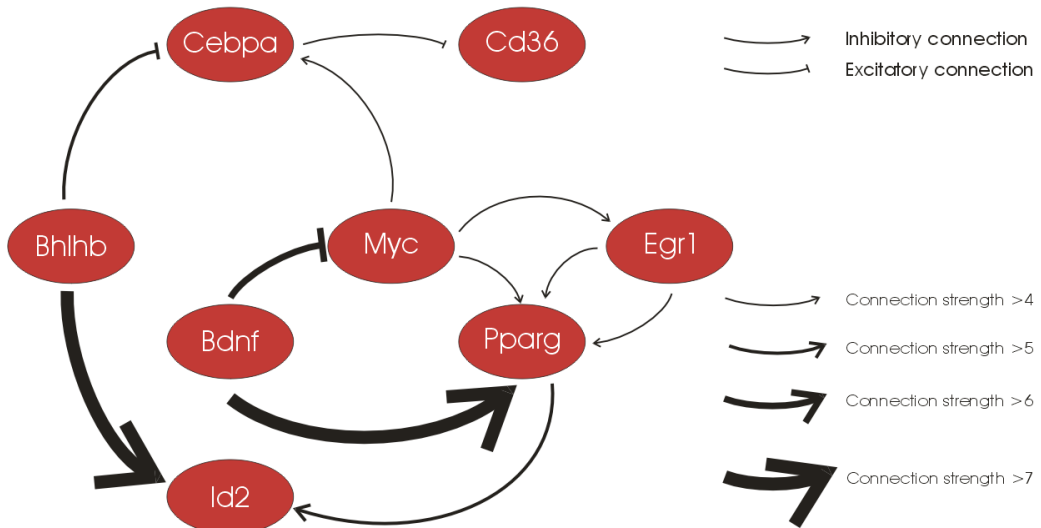


Figure 3.8: EG model - Network determined from the essential genes

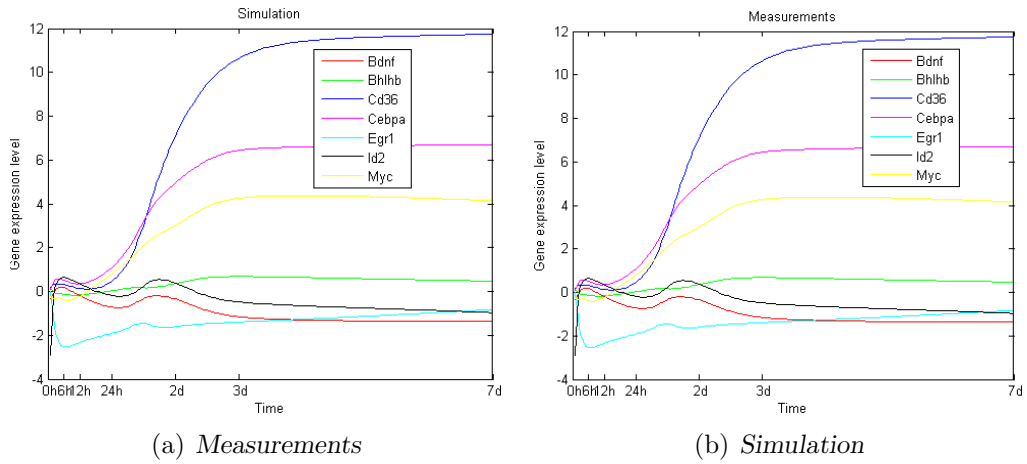


Figure 3.9: EG model: simulated and measured gene expression levels.

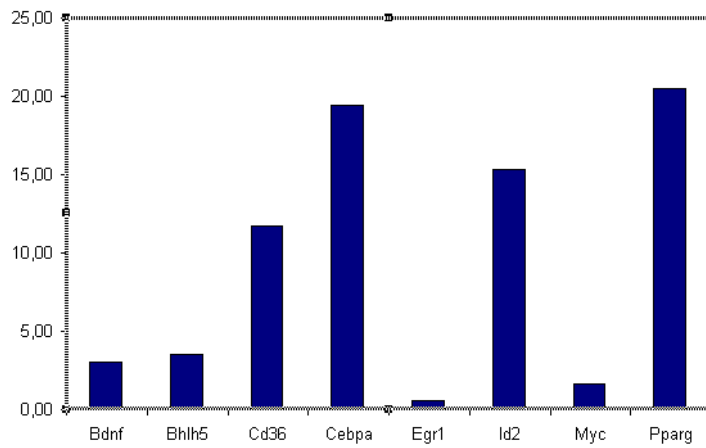


Figure 3.10: EG model - impact of the essential genes measured as the resulted mean squared change

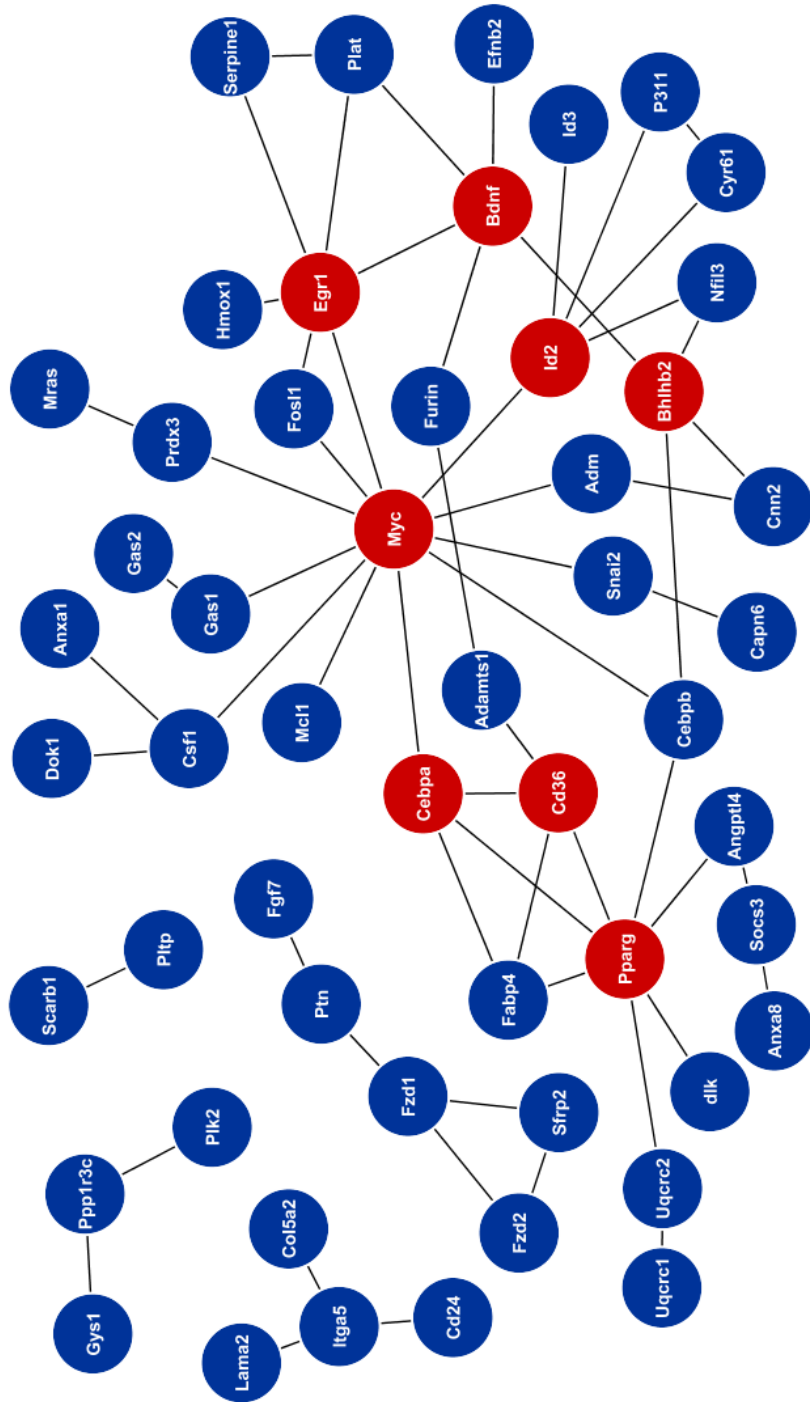


Figure 3.11: Network view of the identified essential genes. The genes selected for modeling are shown on pink circles.

# Chapter 4

## Discussion

The chapter starts with a section dedicated to the results of the ODE model. In the second section the analysis of the results of the echo state model is presented. The last section of this chapter contains the discussion of the results delivered by the "essential" genes model.

### 4.1 Ordinary differential equation model

In this section the results obtained from the ODE model are discussed. First the network's response to the standard adipogenic cocktail is compared to the measured data. Then the response of the network to other input cocktails is compared to available experimental data. These findings are followed by the analysis of the overall performance of the model.

The ODE model (see subsection 2.1.2) was simulated using the standard adipogenic cocktail DMI (see Table 3.1). The results of the simulation and the measured gene expression levels were presented in Figure 6.1 (Appendix) and in Figure 3.1.

The model computed a good approximation of the measured data. The simulated gene expressions did not perfectly match the measured data, but it was obvious that they shared some characteristics. One of the most important similarity was that the gene expression reached a so called "saturated" value that did not drastically change after day four of adipogenesis. In general the forms of the curves were similar to each other.

The gene expressions of *Scd1* and *Fabp4* (Figure 3.1) correlated with the biological understanding of adipocyte markers. Starting with about day four of differentiation, the markers exhibited very high gene expression levels.

The simulated gene expressions of the master regulators *Pparg* and *Cebpa* matched the measured data and as a result, matched the biological beliefs

regarding these regulators.

Due to clarity reasons the simulation results of other factors used in this model were not presented. These results matched the measured data in the same way as Pparg, Cebpa, Scd1 and Fabp4 did.

The network was also simulated using different input cocktails (DM, DI, MI, D, M, I) described in Table 3.1. The results of the simulations of Cebpa were plotted in Figure 3.1 and Figure 6.1. Figure 6.1 can be found in the Appendix. Cebpa was chosen because the protein levels of it were determined in [15] using the same input cocktails. It is clear that the ODE model computes gene expression levels and not protein levels. But here a general comparison is done, not a comparison of the exact numbers.

The results found in [15] were shown in Figure 2.4. The western blots were done on day five of differentiation. Du to this, the gene expression levels on day five of differentiation were used for comparison.

The computed gene expression profiles did not match the results of the western blots. The model predicted high gene expression levels for all the used input cocktails. The western blots experiments showed that the protein levels of Cebpa were high for DMI and DM, but low for all other input cocktails (no difference compared to the control case). That the results for Cebpa matched the western blots results for the DMI case is due to the fact that these were similar to the measured data. This case was discussed before. In case of DM, the simulation results also matched the western blots results (high gene expression levels and high protein levels). In all other cases the simulation results did not match the western blots results.

Looking at Figure 6.1 and Figure 3.1 it can be seen that the response of Cebpa to different input cocktails was almost equal. Only the gene expression levels in the end of the differentiation process were, in some cases, different (see y-axis of the plots). This revealed that the model did not possess a good generalization capacity. Only on one input cocktail, different to the one used to determine the model's parameters, the model computed a result similar to results obtained through other techniques. This is probably due to the fact that the parameters of the model were computed from only one experiment. These parameters are capable of reproducing the data they were computed from. If more measurements were available that could be used for parameter estimation, the performance as well as the generalization capacity of the model would increase.

## 4.2 Echo state network model

In this section the results presented in section 3.2 are discussed. First the results are discussed that were obtained when microarray data was used for training and western blot data was used for testing. Then we turn to the results obtained when the ESN model was trained using leave-one-out cross validation.

First the ESN model was trained on the gene expression data obtained during the microarray experiment. All adipogenesis regulators known from the ODE model were employed. In this way, gene expression levels of 14 regulators were used. The input to the ESN model was the time course of the standard adipogenic cocktail DMI. During the training phase, the gene expression levels of the 14 regulators were almost equal to the measured gene expression levels. In Figure 3.2 the gene expression levels of *Fabp4* and *Scd1* are shown. The same reconstruction quality was found for all other regulators.

The network trained in this way was used to simulate the gene expression levels of the adipogenesis regulators for different input cocktails. The simulated gene expression levels in response to DM were shown in Figure 3.4. It can be seen that the predicted gene expression level of *Cebpa* did not match the measurements but was higher than in the DMI case. This fact corresponds to the current knowledge that that adipogenic cocktail DM (lacking Insulin) induces higher gene expression levels of *Cebpa* compared to the adipogenic cocktail DMI.

In the other cases the performance of the model is not good. It seems that the ESN model adjusts to well to the training data.

In order to use the concept of leave-one-out cross validation, the microarray experiment as well as the derived gene expression levels from the western bolts were employed. In this way seven input/output cases were constructed (see Table 2.2). The simulated gene expression levels were computed for each of the experiments. The output of the ESN model comprised of the gene expression levels of *Fabp4* and *Scd1*.

The best results were obtained when the ESN model was tested on the input cocktail I. This means that the parameters of the ESN model were computed from the input/output data of DMI, DM, DI, MI, D and M and than the the gene expression levels of *Fabp4* and *Scd1* were simulated in response to the input cocktail I. In this case the exhibited generalization quality was very good (see Figure3.5). The model is suited to be used in other biologically interesting scenarios.

A very good generalization performance was also shown for the gene expression levels of *Fabp4* (Figure 6.4). The ESN model was tested on the

adipogenic cocktail D. The simulated gene expression levels of Fabp4 were almost equal to the measured data. This was not the case for Cebpa. In the other cases, the generalization performance was poor (see Figure 6.3 in the Appendix).

The concept of leave-one-out cross validation was successfully applied. Even if only a small amount of data is available, a model was derived that was able to predict the gene expression levels of Fabp4 and Cebpa in response to an adipogenic cocktail that was never "seen" by the model.

### 4.3 Essential genes model

The first two models that were presented made use of well known adipogenesis regulators like Pparg, Cebpa and Cebpb. First, an ordinary differential equation model was implemented. Afterwards, an echo state network model was employed. A totally different strategy was used in the EG model.

The "essential" genes were identified from different experiments. The heat map of the expression profiles was presented in Figure 3.6. A network view of the essential genes was computed and can be seen in Figure 3.11. From this network, the genes having at least four connections were chosen to be employed in the EG model.

The parameters of the model were identified using a genetic algorithm. The computed mean squared error between the measurements and the simulation was very small. The impact of each gene was computed and can be seen in Figure 3.10. Here the mean squared change produced by each gene was shown. Out of the graphic it can be stated which genes generated the highest change compared to the initial simulated profiles. These genes could play an important role in the process of adipogenesis. It is not a surprise, that the highest change is produced by Pparg and Cebpa. The model confirms once again the important role played by this two regulators. The other genes that also induce a high change in the expression profiles are more interesting. These could also be a key players in the process of adipogenesis.

One of the strongest predicted connections was between Bdnf (Brain derived neurotrophic factor) and Pparg. In [32] evidence was presented that shows sensitivity of Bdnf to the overexpression of Cebpb. Since there also exists evidence that Pparg is regulated by Cebpb, the connection between Bdnf and Pparg makes sense. In [30] a study was presented in which gene transfer of Bdnf in mice led to weight loss and to alleviation of obesity related insulin resistance. Another article [20] shows a direct link between eating behavior and Bdnf activity. There are not many studies on Bdnf and on the role this factor plays in adipogenesis, but there is definitely a connection that should

not be ignored.

According to the EG model another important regulator of adipogenesis was Bhlhb. In [37] and [28] it was shown that inhibiting of Pparg is possible through Bhlhb (a transcription factor for the gene Stra13).

A connection between Pparg and Id2 is studied in [19]. According to this article overexpression of Id2 induced expression of Pparg and mice lacking Id2 expression exhibited reduced adiposity. Furthermore the article presented results confirming the role of Id2 in the modulation of Pparg expression.

There also exist studies [33, 34] indicating that Myc was able to inhibit adipocyte differentiation through blocking Cebpb and Cebp d caused activation of Cebpa and Pparg. It was shown that Egr1 and Egr2 (Krox20) have different roles in adipogenesis. While Krox20 is known to play a positive role (see ODE model and [31]), in [17] it was published that adipogenesis is inhibited by ectopic expression of Egr1 and potentiated by knockdown of Egr1.

Cd36 is implicated in the transport of long chain fatty acids. It was also shown that mice in which the corresponding gene was deleted were save from high fat diet induced obesity. In [21] it was claimed and demonstrated that Cebpa, one of the key regulators of adipogenesis, regulates the expression of Cd36.

The list of proposed genes is reasonable from a biological point of view. The predicted connections actually exist. This model offers the possibility of *in silico* testing of biological hypothesis before investing time and money in *in vitro* and *in vivo* testing. In this way *testable hypothesis* can be formulated and the results could be helpful in deciding over further research.

# Chapter 5

## Conclusion

The health risk involved by obesity justifies the need for a mathematical model of adipogenesis. Until now no such model exists and the complicated mechanisms governing this process are far from being elucidated. Here three different mathematical models were proposed. The ODE model was able to reproduce the gene expression profiles of the key adipogenesis regulators. The ESN model first predicted a higher expression of *Cebpa* in the absence of Insulin and afterwards correct expression levels of *Cebpa* and *Fabp4* in response to Insulin. A list of IBMX and DEX regulated genes was developed for the third model. From this list a subset was chosen for modeling. In this way a list of new genes was created, that are sustained by biological studies, and could play an important role in the process of adipogenesis.

The use of mathematical models for complicated processes is justified by the low (nearly zero) costs of such an *in silico* test compared to the *in vitro* and *in vivo* related costs and time. The simplicity of the mathematical model, compared to the complexity of the biological process, makes it easier to have an overview of the whole problem and keeps one from getting lost in numerous details. The complicated the model is (the more factors involved), the more measurements are needed for reliable results. Nevertheless a model is always an abstraction of a real process with the related advantages and disadvantages.

For these three models a relative small amount of measured data was available. If new measurements would be integrated, which is easily possible, the performance and generalization quality of the models would increase. Particularly the third model is aimed to be employed in the simulation of hypothesis that could reveal new insight to the process of adipogenesis. Hypothesis, if sustained by simulation results, could lead to new studies and research ideas.

# Chapter 6

## Appendix

### 6.1 ODE Model - parameters and additional results

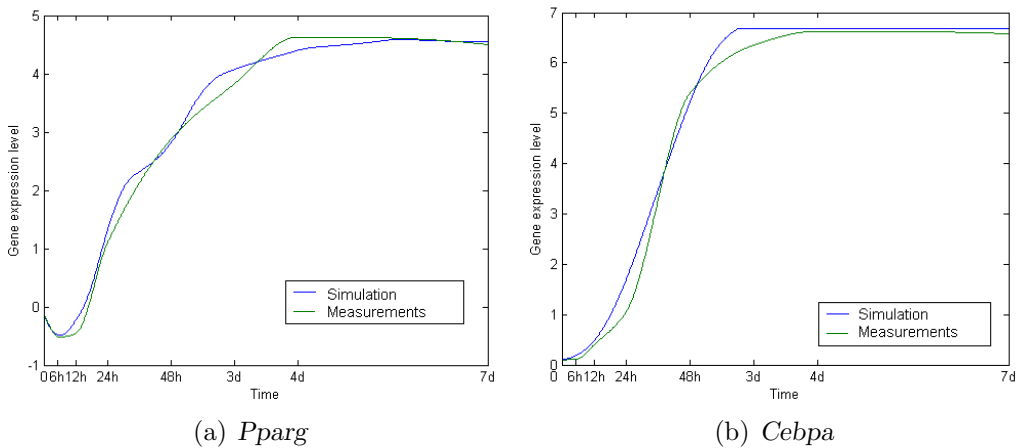


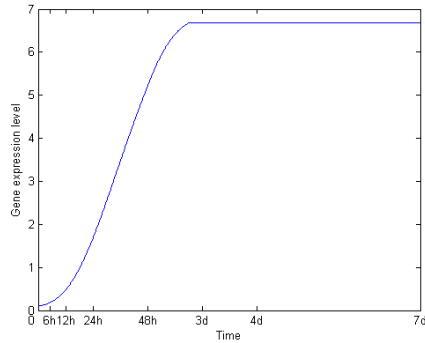
Figure 6.1: ODM model: gene expression levels of master regulators

Events	E1	E2	E3	E4	E5	E6	E7
Simulation time	0	720	2000	3000	4000	6000	8000

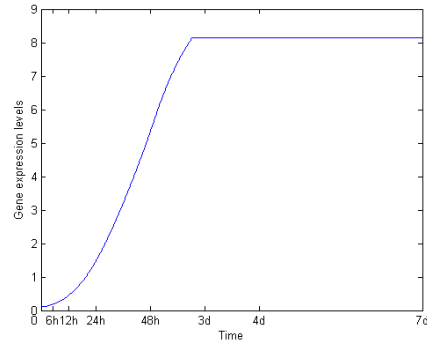
Table 6.1: Events at which the ODE parameters change. The total simulation time was of 10080 which corresponds to 7 days of differentiation with a resolution of 1 minute. The corresponding parameter values were listed in Table 6.2.

	E1	E2	E3	E4	E5	E6	E7
$a_1$	3e-4				0		
$a_2$	18e-4				0		
$a_3$	-3e-4				0		
$a_4$	-54e-4						
$a_5$	-4e-4						
$a_6$	32e-4						
$a_7$	12e-4						
$a_8$	-39e-4						
$a_9$	63e-4						
$a_{10}$	-42e-4						
$a_{11}$	4e-3			1e-9	0		
$a_{12}$	1e-8			1e-5	0		
$a_{13}$	-6e-5				0		
$a_{14}$	-6e-5				0		
$a_{15}$	1e-9						
$a_{16}$	1e-9		2e-3	0			
$a_{17}$	15e-4		0				
$a_{18}$	-9e-9		-5e-3	0			
$a_{19}$	-16e-4		-21e-3	4e-4			
$a_{20}$	3e-4			-15e-5			
$a_{21}$	14e-4		-24e-4		-42e-4		0
$a_{22}$	14e-4						0
$a_{23}$	-15e-4	15e-4		-13e-4	16e-4	-1e-4	-15e-4
$a_{24}$	6e-5	-7e-5		15e-4		0	
$a_{25}$	1e-4					1e-5	
$a_{26}$	1e-5	2e-4				1e-5	
$a_{27}$	1e-5	1e-4			0	1e-7	
$a_{28}$	2e-2	1e-4				1e-7	

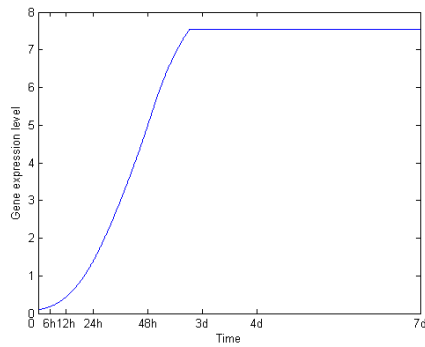
Table 6.2: Computed values of the ODE model parameters. The parameter values change at different events E1 to E7. The events are described in Table 6.1.



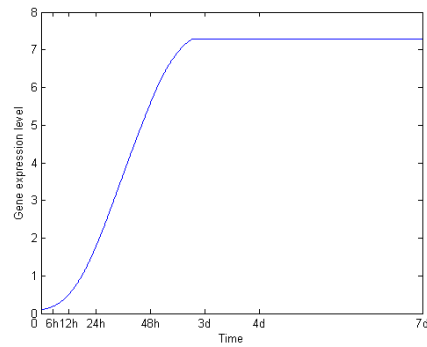
(a) *Input cocktail MI*



(b) *Input cocktail D*



(c) *Input cocktail I*



(d) *Input cocktail M*

Figure 6.2: ODM model: simulated gene expression level of *Cebpa* for different input cocktails

## 6.2 ESN model - sample Matlab code for the implementation of the model using leave-one-out cross validation

```

%% Echo state network model using leave one
%% out cross validation
%% Trains the model and then computes the
%% mean squared error of the model
%% during training phase
%%
close all
clear all
clc

```

```

%load data for the different input cocktails
load('DMI_LOO.mat');
load('DI_LOO.mat');
load('DM_LOO.mat');
load('MI_LOO.mat');
load('I_LOO.mat');
load('M_LOO.mat');
load('D_LOO.mat');

% set initial state of the random generator
% reproducibility of results
rand('state', 1);
randn('state',1);

% define random sequence between each
% input/ouput data

%time steps of random input
rt = 1000;
%random input
ri = randn(size(inputD,1),rt);
%random output
ro = randn(size(outputD,1),rt);
%train time
tt = size(inputD,2);

% total input and output
input = [inputDMI ri inputDI ri inputDM ri ...
         inputMI ri inputI ri inputM ri ...
         inputD ri];
output = [outputDMI ro outputDI ro outputDM ...
         ro outputMI ro outputI ro outputM ...
         ro outputD ro];

%iterating through the 7 input/output cases
for k = 1:1:7
    %training phase

    %preparing input/output data
    [train_input train_output ...

```

```

test_input test_output ...
text data_index fig] = create_in_out_L00 ...
                    (input,output,k,rt,tt);
N = 30;                %units in dynamic reservoir
L = size(train_output,1);    %output units
K = size(train_input,1);    % input units

%weights from the input to the dynamic reservoir
w_scale = 0.9;
connect = 0.1;
C = rand(N,K)<connect ;
C=C.*(2*randint(N,K)-1);
Win = w_scale .* C;

% weights in the dynamic reservoir
W0 = randn(N,N);
[V,D] = eig(W0);
lambda_max = max(max(abs(D)));
W1 = W0./abs(lambda_max);
alpha = 0.9;
W = alpha.*W1;

% weigths from the output units back to the
% dynamic reservoir
connect_back = 0.01;
C_back = rand(N,L)<connect_back ;
C_back = C_back.*(2*randint(N,L)-1);
wb_scale = 0.1;
Wback = wb_scale*C_back;

%training with teacher forcing
net_delay = 2;
noise_scale = 0.01;
X = zeros(size(train_input,2),N);

%for each time step
for i = net_delay+1:1:size(train_input,2)
    noise = randn(1,N)*noise_scale;
    X(i,:) = tanh(Win *train_input(:,i-...
                    (net_delay))+W*X(i-1,:)+...
                    +Wback*train_output(:,i - ...

```

```

        net_delay)+noise');
end

%computing Wout with Bayesian regulation
startM = 1;
m = X(data_index,:);
a = ones(1,size(m,1))';
M = [a m]';
T = output(:,data_index);
net = newff(minmax(M),size(T,1),{'purelin'}...
            , 'trainbr');
net.trainParam.epochs = 50;
randn('seed',11);
net = init(net);
[net tr] = train(net,M,T);
Y = sim(net,M);

%mean squared error
mse_train_cebpa(k) = sum((Y(1,:)-...
train_output(1,data_index)).^(2))/(length(Y(:,1)))
mse_train_fabp4(k) = sum((Y(2,:)- ...
train_output(2,data_index)).^(2))/(length(Y(:,2)))

%test phase
[mse_test_cebpa(k), mse_test_fabp4(k)] = ...
test_ESN_LOO_reg(test_input,test_output,...
net,X,Win,W,Wback,1000,text,fig);
end

%% Computes the mean squared error
%% of the network
%% RETURN VALUES:
%% ec = mean squared error of Cebpa
%% ef = mean squared error of Fabp4
%% INPUT PARAMETERS:
%% u = random input to the ESN
%% d = random output of the ESN;
%% net = network computing the output
%% of the ESN
%% Win = weights from the input units
%% to the dynammic

```

```

%%      reservoir
%% W = weights of the dynamic reservoir
%% Wback = weights from the output
%% units back to the dynamic reservoir
%% r = delay
%% text, fig = used for plotting

function [ec,ef] = test_ESN_LOO_reg(u,...
d,net,X,Win,W,Wback,r,text,fig)

random_input = randn(size(u,1),r);
random_output = randn(size(d,1),r);
u = [random_input u];
d = [random_output d];

for j = 2:1:size(u,2)
    Y(j-1,:) = sim(net,[1 X(j-1,:)]');
    %no teacher forcing is used anymore
    X(j,:) = tanh(Win *u(:,j-1)+W*...
    X(j-1,:)'+Wback*Y(j-1,:))');

end

day5 = 0;
cebpa_esn = Y(r+day5+1:end,1);
cebpa_meas = d(1,r+day5+1:end-1)';
fabp4_esn = Y(r+day5+1:end,2);
fabp4_meas = d(2,r+day5+1:end-1)';

ec = sum((cebpa_esn - cebpa_meas).^2)...
    /(length(cebpa_esn));
ef = sum((fabp4_esn - fabp4_meas).^2)...
    /(length(fabp4_esn));

```

## 6.3 ESN Model - additional results

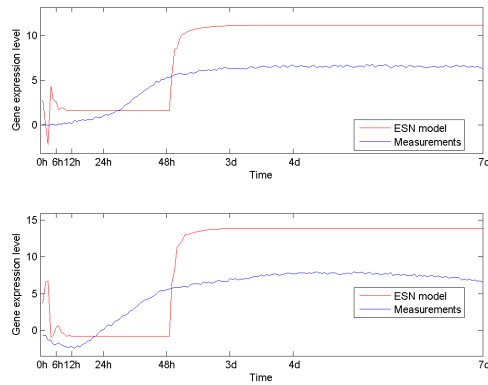


Figure 6.3: ESN model - case DMI using leave-one-out cross validation: gene expression level of *Cebpa* (upper part) and *Fabp4* (lower part) during testing phase

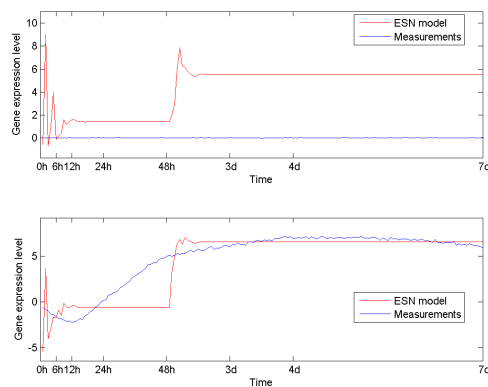


Figure 6.4: ESN model - case D using leave-one-out cross validation: gene expression level of *Cebpa* (upper part) and *Fabp4* (lower part) during testing phase

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