Regulatory behaviour of skeletal muscle thin filaments modeled with Cell-DEVS

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Abstract—**There are six proteins that are involved in muscle contraction: actin, myosin, tropomyosin, troponin C, troponin I and troponin T. The cellular automata model implemented simulates the interactions between the actin sites and a troponin sites (integrated functional units of tropomyosin-troponin complex). The model was executed using Cell-DEVS formalism, which exploits ease of programming while still efficiently executing cellular models. The transition rules were restricted to functions of the neighbouring molecules' states and rate constants.**

Index Terms—**Biological system modeling, Biological systems, Biology computing, Computational modeling, Computer Simulation, Discrete event simulation, Discrete event systems, Formal specifications, Muscles**

I. INTRODUCTION

MUSCLE contractions are dependent on interaction between thick and thin filaments. Thick muscle filaments are primarily composed of myosin, while thin muscle filaments are predominantly composed of actin, tropomyosin (Tm) and troponin (Tn) protein molecules. In a thin filament the stoichiometric relationship between these three protein molecules, actin, tropomyosin and troponin, is 7:1:1 respectively.

Most of the muscle thin filament is made up of globular actin (G actin) proteins. These protein molecules polymerize forming two helical stands known as fibrous actin (F-actin). The troponin molecule is a complex structure that can be broken down into three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT). Tropomyosin proteins bind to each troponin molecule (TnT subunit) to form a tropomyosin-troponin (Tm-Tn) complex.

Each G actin protein encompasses a binding site that allows a myosin head or cross-bridge to bind. However, when the muscle fibre is at rest (i.e. not contracted) these sites are saturated by a number of TnI protein molecules. On the other hand, the TnC binding molecule is calcium ion binding protein; and the TnT molecule binds to the tropomyosin molecule. Furthermore, the Tm-Tn complex regulates the thin

filament component of the muscle contraction that is dependent on calcium ions.

There are numerous models and experiments conducted that examine the regulation of muscle contractions, all of which came to very similar conclusions that is – there is a strong correlation between the various components of the muscle fibre – primarily between the six proteins: actin, myosin, tropomyosin, and troponin C, I and T. Modelling and simulation of these models assist in validation of the complex behaviour of the proposed biological models. In this case specifically, modelling and simulation can assist in thoroughly understanding the regulatory behaviour of muscle fibres. One way to accomplish this is to create a cellular automata (CA) model that depicts the interactions between the protein molecules in the muscle thin filament. The model outlined here is an implementation of a pre-existing model [1] that was developed in the year 1994.

Traditionally, the computation time of CA models is considerably high. This is attributed to one of its unique features, which is the simultaneous application of a set of transition rules for site defined in the entire cellular space. Additionally, another disadvantage of CA models is the use of discrete time, which poses a precision constraint on the model. The work presented here exploits Cell-DEVS (Discrete Event Systems) formalism to solve the fore mentioned problems [2]. Thus, using Cell-DEVS, this report aims to exhibit the complex biological process of regulatory behaviour of muscle thin filaments.

The report is organized in the following manner: to begin with a brief overview of Cell-DEVS and CD++ is presented. This is followed by a description of the model, its specification using Cell-DEVS formalism. This includes: states, initial conditions, definition of neighbourhood, and transition rules. Finally, this report discusses the implementation using CD++ toolkit and the test results.

II. CELL-DEVS AND CD++

DEVS formalism [3] is a continuous time technique whose framework is based on the coupling of models allowing for the construction of modular and hierarchical models. Furthermore it supports the reuse of models, which in turn reduces the time required not only for development but also for testing of

models. The most basic model in DEVS is behavioural or atomic model. The atomic model is defined by a sequence of transitions between sequential states, and its reaction to external input and the generation of output. A number of atomic models or coupled models can be amalgamated forming a hierarchical model called a coupled (structural) model [4].

Cell-DEVS formalism combines DEVS formalism and cellular automata with timing delays. This is achieved by defining each cell as an atomic model and it's neighbourhood as a coupled model. There are two main advantages to Cell-DEVS: ease of programming and efficient execution of cellular models. The latter is achieved by allowing only the active cells to be triggered. Similar to the atomic model in DEVS formalism, each cell in the Cell-DEVS consists of a state variables and a local computing function. The states are updated just like in a traditional cellular automaton – that is based on its current state and the state of the cells in its neighbourhood.

Each cell in Cell-DEVS is a timed DEVS cell atomic model and can be formally described as:

$$TDC = < X, Y, S, N, type, d, \tau, \delta_{int}, \delta_{ext}, \lambda, D >$$

The variable X represents an array of external inputs, and Y represents an array of external outputs. The variable S defines the cell state definition while the variable N defines the relative neighbourhood set. The type of delay (transport – delay in the output of the cell, inertial – delay with pre-emptive schematic, or other – user specified) is defined by the variable $type$. In addition the duration of the delay is defined by the variable d. The evaluation of the future state of the cell is defined by the local computing function τ. The last four variables define the behaviour of the cell. The internal transition function is defined as δ_{int}, while the external transition function defined by the variable δ_{ext}. The variable λ is the output function and the D is the state duration [5].

A Cell-DEVS coupled model represents the entire cell space can be formally described as:

$$GCTD = < X, Y, X_{list}, Y_{list}, \eta, N, \{m, n\}, C, B, Z, select >$$

The variable X represents an array of external inputs, Y represents an array of external outputs, and X_{list} and Y_{list} are the input and output coupling list respectively. The variable η is the number of dimensions in the cell space and N is the relative neighbourhood set. The number of cells in each dimension is defined by the variables $\{m, n\}$. The variable C defines the cells space set, B is the border cell set and Z is the translation function. The $select$ variable is a tie-breaking function for simultaneous events [5].

CD++ is a modelling and simulation toolkit that implements both Cell-DEVS and DEVS formalisms. The toolkit allows for DEVS atomic models to be programmed in C++. Coupled models on the other hand can be programmed using the built in specification language.

III. MODEL DESCRIPTION

There are several models that provide an in-depth analysis of the regulatory behaviour of muscle thin filaments. This paper does not focus on the details of the mechanisms of thin filaments but instead focuses on the implementation of a pre-existing model that is outlined in [1].

The model assumes that each actin site in the thin filament can only be represented by one of two states: active or inactive state. These states denote the binding of myosin S1 to actin. The active state represents a strongly bound myosin S1 molecule. On the other hand the inactive state represents a weak or unbound myosin S1 molecule.

Each troponin site in the model also can be one of two possible states: inhibiting state and facilitating state. Note that the model assumes that the troponin site in the cellular automata model represents the Tm-Tn functional complex. When the troponin sites are in the inhibiting state it inhibits the activation of nearby actin sites, however, when it is the facilitating state it facilitates the activation of the actin sites that are in its neighbourhood. Additionally, the inhibiting state of the troponin site represents the first conformation of the Tm-Tn functional complex. On the other hand the facilitating state of the troponin site corresponds to the second conformation of the Tm-Tn functional complex.

The literature under examination models the muscle thin filament as a one dimensional array of finite automata. This is due to the fact that there has not been any substantial evidence of cooperative interaction between the molecules exists between the stands. However, to test various different cases, a two dimensional finite automata that was implemented instead of a long one dimensional finite automata.

Furthermore, the model modified to test the effects of the rate constants. This is due to the fact that most of the cells will not be active due to the small rate constant in comparison to the other rate constants. That is the rate constants for all the reactions involved in the regulatory behaviour of thin filaments of muscles range from magnitudes of 10^{-2} to 10^{7}. The first way that the model was modified was by removing the rate constants that are dependent on the concentration of the actin or troponin sites. Note that the rate constants that are only dependent on the time step could not be removed because the entire transition was dependent only on the rate constant (i.e. the state change is not a function of the states it's neighbouring cells). Another simulation was created by simply removing the powers of 10. This ensures that all the states have a much better chance of changing during the simulation. Both approximations are not realistic since the relative values of the rate constants are quite important as they account for some key information about the interactions between the molecules.

IV. MODEL SPECIFICATION

The model described above was implemented in Cell-DEVS as a coupled model. There are no inputs or outputs for this model. Hence there is no need of input or output coupling ports. The variable η (i.e. the number of dimensions in the cell

space) was set to 2 to represent a two dimensional cellular automata. The variable N (i.e. the relative neighbourhood set) is defined as the first, seconds, third and seventh cells to the left and right of the cell of interest (See Fig. 1).

Fig. 1. Definition of neighbourhood for muscle thin filament model. Where C represents the cell lattice under consideration; R1,R2,R3 and R7 represents the cell lattice that are first-,second-,third- and seventh-right nearest neighbours respectively; and L1, L2, L3 and L7 represents the cell lattice that are first-,second-,third- and seventh-left nearest neighbours respectively;

The borders for considered to be wrapped (B = 0) since the model dictates that the cellular space has periodic boundary conditions [1].

An active site in the inactive state is denoted by state 1 (s1), while the active state is denoted state 2 (s2). Likewise, the inhibiting state of the Troponin complex is denoted by the state 3 (s3) while the facilitating state corresponds to state 4 (s4).

V. INITIAL CONDITIONS

Because the paper that is being examined does not include a set of initial conditions, this implementation set the initial conditions of the cells randomly. However certain conditions had to be met. The first condition states that for every seven actin site (regardless of its current state) it must have at least one Troponin located in its vicinity. The second condition states that a Troponin molecule must be within 7 to 8 cells of another Troponin molecule.

In addition, because of the lack of information provided by the paper, the original model was simplified in order to allow for more iteration to take place. This allows for the comparison of the effect of rate constants and also allows for the simulation to run further.

VI. TRANSITION RULES

The original model presented is a diffusion driven stochastic model. The state transition rules are a function of a transition probability and or its neighbourhood configuration. The transition probability is expressed in units of concentration and time.

An actin site's is activation is represented by its transition from s1 to s2. This transition can be described by five different rate constants and neighbourhood configurations: $a_{12}^{(i)}, a_{12}^{(f)}, b_{12}, c_{12}, d_{12}$, and e_{12}. Conversely an actin site's deactivation is represented by its transition from s2 to s1. This transition (a_{21}) however is only dependant on a rate constant that is a function of time.

Similar to the transitions defined for the actin sites, the transition of a troponin site from an inhibiting to a facilitating state is represented by s3 to s4. This transition is represents the binding of calcium which is described by three transitions: a_{34}, b_{34}, and, c_{34}. These transitions are also dependant on neighbourhood configurations. On the other hand, the transition that depicts a troponin site to be inhibiting from facilitating (denoted by the state transition s4 to s3), is

independent of neighbourhood configuration and concentration value.

Summary tables (Table I, Table II, and Table III) of the various transition rules are outlined. For a more detailed description of the transition rules, refer to the original article [1].

TABLE I
NEIGHBOURHOOD CONFIGURATION FOR ACTIN SITES

Rate Constant	Neighbourhood configuration						
	L3	L2	L1	C	R1	R2	R3
$a_{12}^{(i)}$	4	1	1	1	1	1	*
	*	4	1	1	1	1	*
	*	1	4	1	1	1	*
	*	1	1	1	4	1	*
	*	1	1	1	1	4	*
	*	1	1	1	1	1	4
$a_{12}^{(f)}$	3	1	1	1	1	1	*
	*	3	1	1	1	1	*
	*	1	3	1	1	1	*
	*	1	1	1	3	1	*
	*	1	1	1	1	3	*
	*	1	1	1	1	1	3
b_{12}	*	1	2	1	1	1	*
	*	1	1	1	2	1	*
c_{12}	*	*	2	1	1	*	*
	*	*	1	1	2	*	*
d_{12}	*	*	2	1	2	*	*
e_{12}	*	2	1	1	1	*	*
	*	*	1	1	1	2	*
a_{21}	*	*	*	2	*	*	*

'C' denotes the cell that is under consideration; L1, L2, and L3 represent the cell lattice that are the first-, second-, and third-left nearest neighbours respectively; R1, R2, and R3 represent the cell lattice that are the first-, second-, and third-right nearest neighbours respectively.
The symbol '*' denotes that the related site maybe either an actin site in state 1 or 2, or a troponin site in state 3 or 4 (adapted from [1]) .

TABLE II
NEIGHBOURHOOD CONFIGURATION FOR TROPONIN SITES

Rate Constant	Neighbourhood configuration								
	L7	L3	L2	L1	C	R1	R2	R3	R7
a_{34}	3	*	*	*	*	*	*	*	3
b_{34}	4	2	*	*	3	*	*	*	*
	4	*	2	*	3	*	*	*	*
	4	*	*	2	3	*	*	*	*
	4	*	*	*	3	2	*	*	*
	4	*	*	*	3	*	2	*	*
	4	*	*	*	3	*	*	2	*
	*	2	*	*	3	*	*	*	4
	*	*	2	*	3	*	*	*	4
	*	*	*	2	3	*	*	*	4
	*	*	*	*	3	2	*	*	4
	*	*	*	*	3	*	2	*	4
	*	*	*	*	3	*	*	2	4
c_{12}	4	1	1	1	3	1	1	1	*
	*	1	1	1	3	1	1	1	4
a_{43}	*	*	*	*	4	*	*	*	*

'C' denotes the cell that is under consideration; L1, L2, L3 and L7 represent the cell lattice that are the first-, second-, third- and seventh-left nearest neighbours respectively; R1, R2, R3 and R7 represent the cell lattice that are the first-, second-, third- and seventh-right nearest neighbours respectively.

The symbol '*' denotes that the related site maybe either an actin site in state 1 or 2, or a troponin site in state 3 or 4 (adapted from [1]) .

TABLE III
TRANSITION RATE CONSTANT FOR ACTIN AND TROPONIN SITES

Rate Constant	Value
$a_{12}^{(i)}$	1.176×10^1 M^{-1} step^{-1}
$a_{12}^{(f)}$	1.000×10^3 M^{-1} step^{-1}
b_{12}	4.546×10^4 M^{-1} step^{-1}
c_{12}	7.692×10^3 M^{-1} step^{-1}
d_{12}	5.556×10^3 M^{-1} step^{-1}
e_{12}	1.786×10^3 M^{-1} step^{-1}
a_{21}	1.000×10^{-2} step^{-1}
a_{34}	4.348×10^6 M^{-1} step^{-1}
b_{34}	1.538×10^7 M^{-1} step^{-1}
c_{34}	7.143×10^6 M^{-1} step^{-1}
a_{43}	5.000×10^{-1} step^{-1}

Table adapted from [1].

VII. IMPLEMENTATION

The original and modified models outlined above were implemented using the CD++ toolkit. The transition rules defined in section VI were implemented. For the original model, it should be noted that the rate constants were normalized because the rate constants that are involved in the transition rules vary by 9 powers of 10 magnitudes. The rate constants were implemented by generating number between 0 and 10,000,000,000 in a uniform distribution and comparing the normalized rate constant of a transition rule to the number that was generated. An example of a rule that was implemented is:

```
rule : 2 100 {(0,0) = 1 and (0,-2) = 1
and (0,-1) = 2 and (0,1) = 1 and (0,2) =
1 and uniform(0,10000000000) < 4546000}
```

For the extended model, the rules were modified not to include the rate constants with the exception of the transition between active to inactive state of the actin sites and the facilitating to inhibiting state for the Troponin complex sites. This is because these two transitions were only dependant on its corresponding rate constant and were only a function of time steps and not a function or their respective molar concentrations.

In addition in order to see the model visually the Cell-DEVS animation was used to draw the results. A colour palette was also created using this tool in order to enhance visual impact. The colours chosen were the same as those outlined in the original paper. That is green representing the inactive actin site, red representing the active actin site, and black and yellow representing the inhibiting and facilitating Troponin site respectively.

VIII. TEST RESULTS

The paper did not display any results of initial conditions; hence the model could not be verified. However, each rule and model was tested individually. It should be noted that all the rate constants were taken into consideration assuming each cell contained 1M of the its contents. In other words, each actin site was represented by 1M of actin in the active or inactive site. Similarly, each cell of the Tm-Tn complex also had the same concentration of 1M. An example of the implementation can be seen in Fig. 2.
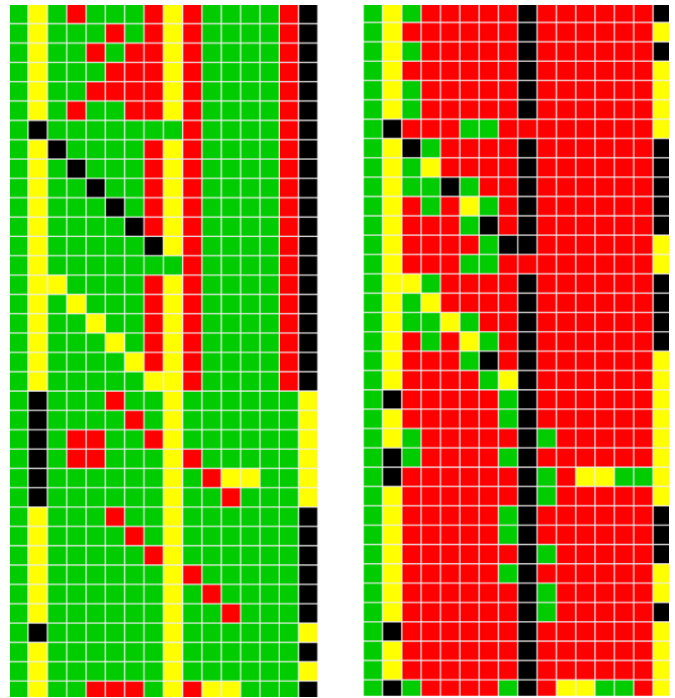

Fig. 2. Snapshot from the simulation. The snapshot of the left shows the initial conditions of the muscle thin filament and the right is a snapshot when t=t+1.

Comparing the different versions of the model, it should be noted that the rate constants contain important information about the interactions between molecules, however they also dictate the length of time that the simulation can be run for while state changes occur. Thus the modified versions although ran for longer period of time, it does not depict reality.

IX. CONCLUSION

A model that depicts the regulatory behaviour of muscle thin filaments was specified using Cell-DEVS formalism and then implemented using the CD++ toolkit. The Cell-DEVS formalism was easy to understand and implement, with the exception of implementation of rate constants. Additionally, it should be noted that this paper did not concentrating in validating or optimizing the model.

Possible future enhancements include, but are not limited to the verification of the model, optimizing the implementation of rate constant, adaptation of the model in 2-dimensional and 3-dimensional space. In addition a better way to visually

represent a long thin filament would also prove to be beneficial. Lastly, the model could be modified to incorporate other state in order to help distinguish other important behaviours. This includes the creation of another state to distinguish between a weak and unbound myosin S1 molecule. Lastly, this model only models a single fibre; however in reality tons of these fibres make up a motor unit.

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