Palytoxin Inhibits the Sodium-Potassium Pump – An Investigation of an Electrophysiological Model Using Probabilistic Model Checking

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Abstract. Automatic verification techniques such as Probabilistic Model Checking (PMC) have been successfully applied in the specification and analysis of stochastic systems. Some biological systems show these characteristics, allowing PMC usage in unexpected fields. We present and analyze a probabilistic model for palytoxin toxin (PTX) effects on cell transport systems, structures which exchange ions across the plasma membrane. Several diseases are linked to their irregular behavior and their study could help drug development. The model developed in this work shows that as sodium concentration increases, PTX action enhances, suggesting that individuals with diets high in sodium are more vulnerable to PTX. An opposite effect is observed when the potassium concentration increases. PMC can help significantly in the understanding of how cell transport systems behave, suggesting novel experiments which otherwise might be overlooked by biologists.

Keywords: Probabilistic Model Checking, Systems Biology, Sodium-Potassium Pump, Palytoxin, Ion Channels Blockers and Openers

1 Introduction

Probabilistic model checking (PMC) is an automatic procedure to model and analyze non-deterministic, stochastic and dynamical systems. PMC completely explores a probabilistic model, establishing if given properties in special types of logics are satisfied by the model. Different properties can be expressed, such as "What is the probability of an event happening?", which offers important information about the model [7, 20, 16].

PMC can be used to study biological systems, which show some of the characteristics of PMC models. The PMC approach can obtain a better understanding of these systems than others methods are able to, such as simulations, which present local minima problems that PMC avoids [9, 15, 14, 8, ?].

We present and analyze a PMC model of the sodium-potassium pump (or Na^+/K^+-ATPase), a transmembrane ionic transport system that is a fundamental part of all animal cells and plays an important role in several biological functions such as cell volume control and heart muscle contraction. The Na^+/K^+-ATPase is one of the main targets of toxins and drugs and it is related to several diseases and syndromes [3]. In this work we present a model where the pump was exposed to a deadly toxin called palytoxin (PTX) — which completely alters the behavior of the pump — in order to understand PTX effects on the pump [22].

The model has shown that high doses of sodium could enhance PTX effects. For example, when the sodium concentration is increased by 10 times its normal values, the probability of being in PTX related states is increased by 17,46%. This suggests that individuals with electrolyte disturbances (changes in normal sodium or potassium levels caused by diseases or syndromes) are more susceptible to the toxin.

The opposite behavior is observed regarding high doses of potassium – when the potassium concentration is increased by 10 times its normal values, PTX effects are reduced by 23,17%. Both results suggest that sodium and potassium levels could be changed to reduce PTX effects on the pump by decreasing sodium and increasing potassium. Since electrolyte levels in the blood can be manipulated up to a certain degree, the study of their role and capability to change Na^+/K^+-ATPase behavior is important.

Our results show that PMC can improve the understanding of cell transport systems and its behavior and may help in the development of new drugs.

This paper is part of an ongoing effort to better understand transmembrane ionic transport systems. The PMC model of the pump was described in [9], where the dynamics of the pump were studied. The toxin palytoxin was included in the model in [4], where different scenarios for the pump disturbances caused by the toxin in cell energy related reactions were studied.

Outline. In Section 2 we describe ionic pumps. The related work of the analysis of these systems and PMC usage are covered in Section 3. Our model is presented in Section 4 and 5, while our experiments, properties and results are shown in Section 6. Finally, our conclusions and future works are in Section 7.

2   Transmembrane Ionic Transport Systems

Every animal cell contains structures named transmembrane ionic transport systems, which exchange ions from the intra to the extracellular medium. An electrochemical gradient is created due to charge and concentration differences between ions in both sides. Gradient maintenance is conducted by cell transport systems, and without it the cells would not perform their functions properly [2].

Transmembrane transport systems are divided in two types: ion channels (passive transport) which do not consume energy; and ionic pumps (active transport) which consume cell energy (Adenosine Triphosphate or ATP).

The behavior of an ion channel depends on transported ion concentration gradients and moves ions in favor of their gradient. Ionic pumps move ions against their electrical charge, concentration gradient, or both [17].

Only specific ions such as sodium, potassium and calcium can pass through ion channels and ionic pumps. Ionic pumps can be viewed as two gates, one internal and another external, that open or close based on chemical and electrical signals, and other different factors [2].

The sodium-potassium pump (Na^+/K^+-ATPase), exchanges three sodium ions from the intracellular medium for two potassium ions from the extracellular medium (Figure 1). This pump can be in two different states: open to either its internal or external side. When the pump is open to its internal side, three sodium ions can bind to it. An ATP molecule binds to the pump, which is followed by its hydrolysis (or energy consumption), releasing the sodium ions to the external side. An Adenosine Diphosphate (ADP) molecule is released while a phosphate molecule remains bound to the pump. Two potassium ions in the external side bind to the pump, which are released in the internal side. The phosphate is also released. The pump is ready now to repeat the cycle [2].
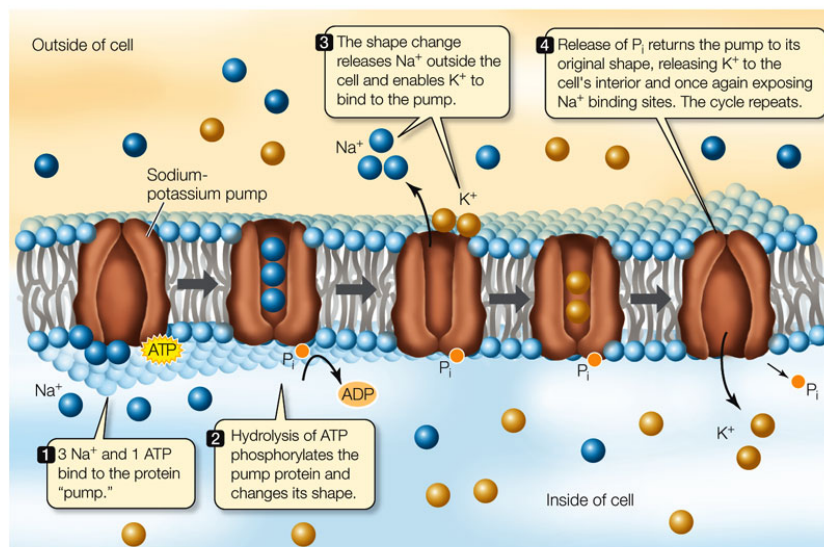
Fig. 1. The Na^+/K^+-ATPase cycle. Adapted from [24].

Ion channels and ionic pumps play an important role in cellular volume control, nerve impulse, coordination of heart muscle contraction, release of accumulated calcium in the sarcoplasmic reticulum for performance of muscle contraction, and several other biological processes [2]. Their irregular behavior is

associated with several diseases, such as hypertension and Parkinson's disease. This makes cell transport systems one of the main targets in research for discovery and development of new drugs [2].

Due to their major role in nervous functions, ion channels and ionic pumps are the main targets of neurotoxins [2]. Palytoxin (PTX), a deadly toxin from the coral *Palythoa toxica*, is an example of a toxin that can affect ionic pumps. PTX changes the behavior of the Na^+/K^+-ATPase, essentially turning it into an ion channel, which means that the pump transfers ions based on their concentration gradient, instead of exchanging ions slowly against their concentration gradient [3].

Despite the discovery of ion channels and ionic pumps over 50 years ago, they are not yet completely understood [2]. However, recent studies about PTX effects on the Na^+/K^+-ATPase are changing how these structures are viewed by the scientific community, helping to understand better how they work [3].

Cell transport systems usually are investigated through expensive and time-consuming experimental procedures in laboratories. Different types of simulations, mathematical and computational methods are also employed to improve the understanding of these structures. Ordinary differential equations (ODE) and Gillespie's algorithm for stochastic simulations are among the methods used for this end [10]. However, despite their ability to obtain valuable information, simulations are not capable of covering every possible situation, and might incur local minima of the model state space, therefore possible overlooking some events, such as ion depletion, where all ions of a cell side have been transferred to the other side.

3   Related Work

3.1   Experimental and Simulational Techniques

Previous researchers have investigated PTX and its interactions with the Na^+/K^+-ATPase [3]. They found that PTX drastically modifies the nature of the pump after binding to it, which changes the behavior of the pump to the one of an ion channel. They suggest that PTX could be an useful tool in experiments to discover the control mechanisms for opening and closing the gates of ion pumps. Rodrigues and co-workers [22] have also discussed this through mathematical simulations using non-linear ODEs and considering only states and reactions related to sodium and potassium exchange.

Interactions of PTX with the complete model for the Na^+/K^+-ATPase are analyzed in [23]. The series of works by Rodrigues and co-workers can be viewed as simulational approaches of the experimental results of [3].

The specific sodium-potassium pump present in cardiac cells is examined in [18] using different models of ODEs. Initially a thirteen state model is presented, however a reduction for the model containing only four states is obtained. It is demonstrated the central role of the sodium-potassium pump in maintaining the cellular concentration levels of calcium ions, essential for the cardiac muscle

contraction. Also, a model is presented for the pump coupled with states and reactions related to cesium, a substance used to perform experiments, which interfere in the behavior of the pump in the same way as drugs and toxins.

3.2   Model Checking

The tools used in the formal verification of biological systems that are more closely related to this work are PRISM [16], BioLab [8], Ymer [26], Bio-PEPA [6] and SPiN [12].

PRISM supports different types of models, properties and simulators [16]. It has been largely used in several fields, i.e. communication and media protocols, security and power management systems. We have used PRISM in this work for several reasons, which include: exact PMC in order to obtain accurate results; Continuous-time Markov Chain (CTMC) models, suited for our field of study; rich modeling language that allowed us to build our model; and finally property specification using Continuous Stochastic Logic (CSL), which is able to express qualitative and quantitative properties.

Clarke and co-workers [8] have introduced a new algorithm called BioLab. Instead of building explicitly all states of a model, the tool generates the minimum number of necessary simulations, given error bounds parameterized for acceptance of false positives and false negatives of the properties to be verified. This algorithm is based on the works of [26], author of the approximate model checker Ymer. We did not use BioLab or Ymer because our initial analysis demanded exact results. Only after these preliminary results we could have used an approximate analysis.

In [27] the authors compare numerical and statistical methods for PMC, since exact model checking is not always possible due to timewise and computational resources restrictions. Therefore, approximate model checking is an alternative when it is acceptable to lose exact results that demand prohibitive execution time in order to obtain approximate results that are obtained in a timely manner. Ymer uses this technique.

The authors illustrate in [15] the application of PMC to model and analyze different complex biological systems for example the signaling pathway of Fibroblast Growth Factor (FGF), a family of growth factors involved in healing and embryonic development. The analysis of other signaling pathways such as MAPK and Delta/Notch can be seen in [14].

The use of PMC is demonstrated also in [13], where the authors examine and obtain a better understanding of mitogen-activated kinase cascades (MAPK cascades) dynamics, biological systems that respond to several extracellular stimuli, i.e. osmotic stress and heat shock, and regulate many cellular activities, such as mitosis and genetic expression.

4   The Na^+/K^+-ATPase Model

Our Na^+/K^+-ATPase model is written in the PRISM language (used by the PRISM model checker) and consists of modules for each of the molecules (Sodium

or Na and Potassium or K) and one main module for the pump. This first part of the model does not include palytoxin, which is later included in Section 5. A fragment of the model is shown in Figure 4, and its complete version can be found in the supplementary material [1]. The complete model has 409 lines and 11 reactions.

```
Na⁺/K⁺-ATPase PRISM Model

module na
  naIn  : [0..(NI+NO)] init NI;        // Number of Na inside cell
  naOut : [0..(NI+NO)] init NO;        // Number of Na outside cell

  // reaction 2: 3naIn + E1 <-> NA3E1
  [r2]  naIn>=3          -> pow(naIn,3) : (naIn'=naIn-3);
  [rr2] naIn<(NI+NO-3) -> 1            : (naIn'=naIn+3);
endmodule

module pump
  E1    : [0..1] init 1;               // e1 conformational state
  NA3E1 : [0..1] init 0;               // e1 bound to three sodium ions

  // reaction 2: 3naIn + E1 <-> NA3E1
  [r2]  E1=1 & NA3E1=0 -> 1 : (E1'=0) & (NA3E1'=1);
  [rr2] E1=0 & NA3E1=1 -> 1 : (E1'=1) & (NA3E1'=0);
endmodule

// base rates
const double r2rate  = 2.00*pow(10,2)/(0.001*V*AV);
const double rr2rate = 8.00*pow(10,2);

// module representing the base rates of reactions
module base_rates
  [r2] true -> r2rate  : true;
  [rr2] true ->rr2rate : true;
endmodule
```

Fig. 2. Na⁺/K⁺-ATPase PRISM Model

Each molecule module contains a variable to store the current number of molecules (i.e. `naIn` for $[Na^+]^i$) and transitions that represent chemical reactions, which are responsible for changing the number of molecules. The concentration of sodium, potassium and palytoxin is discretized as described below in Section 4.1. A list of reactions can be found in [22] and in the supplementary material [1]. Reactions which involve more than one element of each type have to take into account the law of mass action as described below in Section 4.1.

The main module controls the pump, controlling its current state. The states are a boolean vector, where only one position can and must be true. The main module also has transitions which change the pump state.

The Albers-Post model [19] is a kinetic model that represents the Na⁺/K⁺-ATPase cycle (Figure 3 - left side). Its translation to the PRISM language is straightforward. According to it, the pump can be in different states, which change depending on different reactions involving sodium or potassium. The pump can be open or closed to the extra and intracellular sides. Two or three

sodium ions can bind to the pump when it is open to its intracellular side. Two potassium ions can bind to the pump when it is open to its intracellular side. The reactions are bidirectional and their rates were obtained in [22].

In our model, the pump can be in five states: open to its inner side (E1, in our PRISM model); open to its outer side (E2); open to its inner side, with three sodium ions bound to it (NA3E1); closed to both sides with two sodium ions bound it (NA2E2); closed to both sides with two potassium ions bound it (K2E2).
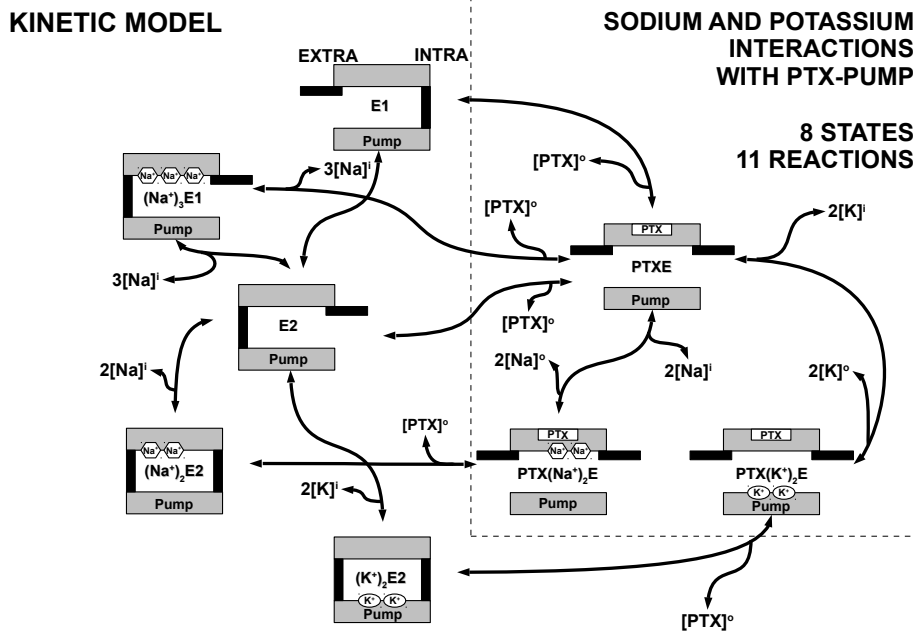

Fig. 3. The classical Albers-Post model [19], where $[Na^+]^i$ and $[Na^+]^o$ are the intra and extracellular sodium (Na) concentrations, $[K^+]^i$ and $[K^+]^o$ are the intra and extra cellular potassium (K) concentrations, and $[PTX]^o$ is the palytoxin (PTX) concentration. Adapted from [21].

4.1 Discrete Chemistry

The main components of our model are molecules (sodium and potassium) and the Na^+/K^+-ATPase, which can interact with each other through several elementary reactions. There is one additional molecule (PTX) in the palytoxin extension for this model, discussed below.

The concentration of each of these components is a discrete variable, instead of a continuous function. Therefore, we have converted the amount of initial concentration of molecules from molarity (M) to number of molecules. The stochastic

rates for forward and backward transitions are from [21]. The substrates concentrations ($[Na]^i = 0.00500$, $[K]^i = 0.00495$, $[Na]^o = 0.00006$ and $[K]^o = 0.00495$) are from [5]. The *cell volume* is from [11].

In order to convert the initial amount of molecules given in molarity ($[X]$) into quantities of molecules ($\#X$), we have used the following biological definition [2]:

$$\#X \ = \ [X] \ \times \ V \ \times \ N_{\mathrm{A}} \tag{1}$$

where V is the cell volume and N_{A} is the Avogadro constant.

The law of mass action states that a reaction rate is proportional to the concentration of its reagents. Therefore, we take into account the reagent concentrations in our model. Considering the discrete chemistry conversion discussed and the palytoxin binding to the pump:

$$E_1 \ + \ \mathrm{PTX} \ \overset{rp_1'}{\rightleftharpoons} \ \mathrm{PTX} \sim E \tag{2}$$
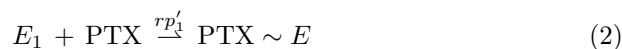
the final rate rp_1 is given as follows:

$$rp_1 \ = \ rp_1' \ \times \ \#(E_1) \ \times \ \#(\mathrm{PTX}) \tag{3}$$

We have used the construct `pow(x,y)` from PRISM to represent the law of mass action. For example, a reaction involving three sodium ions would have a transition rate `pow(naIn,3)`.

5   The Palytoxin Model

The palytoxin model is an extension of the Na^+/K^+-ATPase model described above. It is represented in Figure 3 at the right side, within the dashed boundary. It is based on the description by [22] and [3].

One molecule module (palytoxin) was added to this expanded model, as well as additional states for the pump module and additional reactions for each of the already present modules. Initial concentrations for $[\mathrm{PTX}]^o$ and stochastic rates for transitions between states are from [22]. A fragment of the model is shown in Figure 5 and its complete version can be seen in the supplementary material [1].

The states correspond to the pump bound to PTX, when the pump is open to both sides behaving like an ion channel. There are three additional states for the pump: bound to a PTX molecule (`PTXE`, in our model); bound to a PTX molecule, with two sodium ions bound to their binding sites (`PTXNA2E`); and bound to a PTX molecule, with two potassium ions bound to their binding sites (`PTXK2E`).

6   Results

6.1   Parameters and Model Complexity

Our model can be explored in six different dimensions: extracellular PTX concentration ($[\mathrm{PTX}]^o$), intra and extracellular sodium concentrations ($[Na^+]^i$ and

Palytoxin PRISM Model

```
module ptx
  ptxOut : [0..(PTXO+1)] init PTXO;   // Number of PTX outside the cell

  // reaction p1: PTXo + E1 <-> PTXE
  [rp1]  ptxOut>=1        -> ptxOut : (ptxOut'=ptxOut-1);
  [rrp1] ptxOut<(PTXO-1) -> 1       : (ptxOut'=ptxOut+1);
endmodule

module pump
  PTXE  : [0..1] init 0;                 // non selective pump bound to ptx

  // reaction p1: PTXo + E1 <-> PTXE
  [rp1]  ptx!=0 & E1=1 & PTXE=0 -> 1 : (E1'=0) & (PTXE'=1);
  [rrp1] ptx!=0 & E1=0 & PTXE=1 -> 1 : (E1'=1) & (PTXE'=0);
endmodule

// base rates
const double r1rate = 1.00*pow(10,2);
const double rr1rate = 0.01;

// module representing the base rates of reactions
module base_rates
  [rp1] true -> rp1rate : true;
  [rrp1] true -> rrp1rate : true;
endmodule
```

Fig. 4. Palytoxin PRISM Model

$[Na^+]^o$, respectively), intra and extracellular potassium concentrations ($[K^+]^i$ and $[K^+]^o$, respectively) and pump volume. Each dimension can be modified (increased or decreased) to affect one aspect of the model, which impacts directly to model complexity regarding the number of states, transitions, topology, model build time and property verify time.

Table 1 shows the changes in these values in function of different scenarios. In the Control scenario, $[Na^+]^i = 22$ mM, $[Na^+]^o = 140$ mM, $[K^+]^i = 127$ mM, $[K^+]^o = 10$ mM, $[PTX]^o = 0.001$ μm and the pump volume is 10^{-22} L. In the High Sodium scenario, sodium concentrations are increased 10 times, therefore $[Na^+]^i = 220$ mM, $[Na^+]^o = 1400$ mM, while the other parameters remain unchanged. Finally, in the High Potassium scenario, potassium concentrations are increased 10 times, which changes only potassium to $[K^+]^i = 1270$ mM, $[K^+]^o = 100$ mM.

The columns T_{Build}, T_{State} and T_{Rate} refer to the time to build the model, and to check a state and a transition reward properties. The experiments have been performed in a Intel(R) Xeon(R) CPU X3323, 2.50GHz which has 17 GB of RAM memory.

The standard animal cell volume is 10^{-12} L [11], which is prohibitive to represent in PMC since it would cause the classical problem of state space explosion. Our analysis is restricted to only one cell pump. As a consequence, it would also not be realistic to model the whole cell volume since it is shared between several pumps and other cellular structures. Our abstraction reduces the cell volume focusing our analysis in one or few pumps and their surroundings. We achieve this

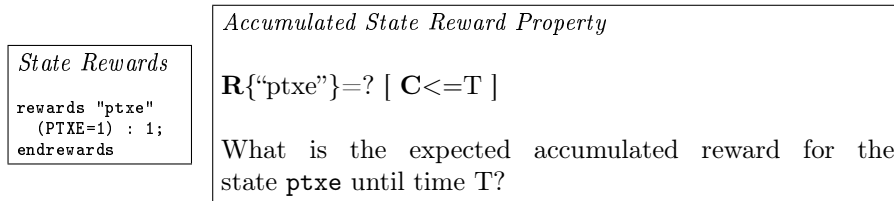| Scenario	States	Transitions	T_{Build}	T_{State}	T_{Rate}
Control	208	652	0.094 s	45.123 s	19.307 s
High Sodium	1880	6020	7.101 s	344.578 s	266.436 s
High Potassium	1274	7140	0.081 s	358.842 s	346.707 s

Table 1. Model complexity, build and check time for different scenarios.

by maintaining the proportions between all interacting components. Therefore, our dimension for cellular volume is called pump volume and is usually 10^{-22} L. Even though those values are many orders of magnitude smaller than the real values, they still represent proper pump behavior, and can be interpreted as using a magnifying glass to investigate a portion of the cell membrane.

On the other hand, for some dimensions we have used more values than intuition suggests, ranging from three orders of magnitude below and above their literature reference values, shown in Section 4.1. This is particularly interesting because we can model different situations for pump behavior, including abnormal concentrations levels for $[Na^+]^i$ due to some disease or syndrome, and different degrees of exposure to $[PTX]^o$, from mild to fatal exposure.

Due to space limitations we have chosen to present the most important properties that we have formulated: state and transition rewards (Sections 6.2 and 6.3). There are also species depletion (reachability) properties (Section 6.4). These and other properties can be seen in the supplementary material [1].

6.2   High [Na$^+$] Enhances PTX action

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State Rewards

```
rewards "ptxe"
  (PTXE=1) : 1;
endrewards
```

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Accumulated State Reward Property

$\mathbf{R}\{\text{"ptxe"}\}=?\ [\ \mathbf{C}{<}{=}T\ ]$

What is the expected accumulated reward for the state `ptxe` until time T?

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Fig. 5. State Rewards and Accumulated State Reward Property

States and rates of the model can be quantified through rewards, a part of PRISM language. One reward for each state and rate is created. Rewards are incremented each time its conditions are true. After calculating each reward we are able to determine state and rate probability. Figure 5 shows the rewards for state `PTXE`, the pump open to both sides and bound to a PTX molecule. Rewards for rates are nearly identical.

Now that the model has rewards for each state and rate, we are able to calculate the expected accumulated reward associated with each state and rate over time, with properties such as the one shown in Figure 5. The **R** operator allows

us to quantify the reward for some given event, for example the number of times the model was in state PTXE. The operator $\mathbf{C}$ allows to quantify accumulated rewards for a given time T, therefore we are able to observe rewards over time.
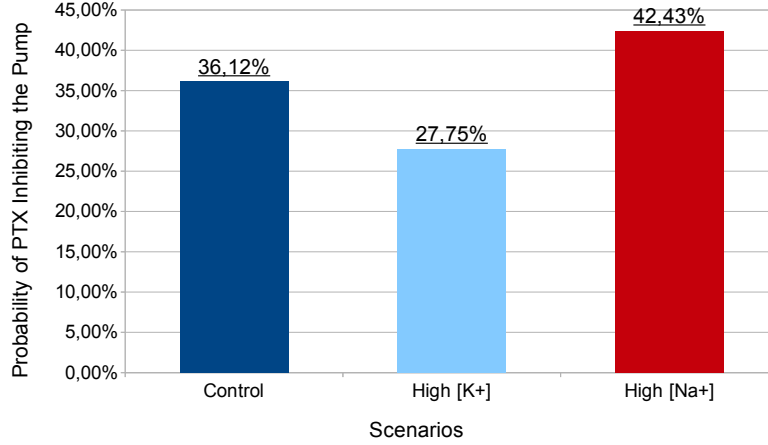

Fig. 6. Probability of PTX Inhibiting the Pump for Different Scenarios in 100 seconds: Control (normal ion concentration); High [K$^+$] scenario (10 times more potassium than normal) which reduces PTX effect by 23,17%; and High [Na$^+$] scenario (10 times more sodium than normal) which enhances PTX effect by 17,46%.

Considering a single pump, a pump volume of 10^{-22} L, a Control scenario (described in the previous subsection), at instant T=100, the expected rewards associated with the state PTXE is 36.2195. In other words, in 100 seconds, the PTX inhibits the pump 36,11% of the time. In a High [Na$^+$] scenario, the expected reward associated with PTXE changes to respectively 45.3599, 42.42% of the time. Therefore, as we increased [Na$^+$], the likelihood of the pump to be bound exclusively to PTX increased 17,46%. This result can be seen in Figure 6, which represents the probability of PTX inhibiting the pump for our three different scenarios, and also its time series version in Figure 7.

This result suggests that sodium enhances PTX action, and as consequence people with electrolyte disturbances would be more vulnerable to this toxin. Sodium disturbances appears in different forms (i.e. hypernatremia) and have different causes, such as diabetes insipidus, Conn's syndrome and Cushing's disease [25]. Sodium concentration could be reduced in order to reduce PTX action. However, this is a solution to be taken with caution since sodium is necessary for survival and its absence would shut down the pump. This is particularly interesting since PTX is found in marine species, which inhabit an environment with a high sodium concentration.
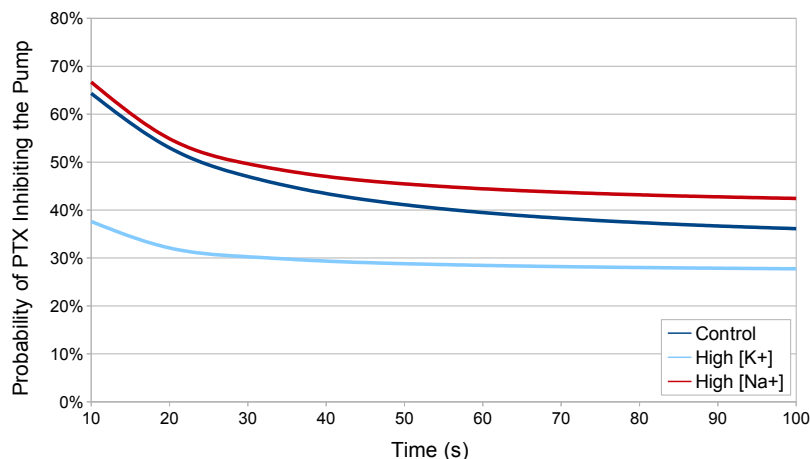
6.3   High [K$^+$] Inhibits PTX action

Fig. 7. Probability of PTX Inhibiting the Pump Time Series for Different Scenarios: Control (regular ions concentrations); High [K$^+$] scenario (10 times more potassium than regular concentration) which reduces PTX effect; and High [Na$^+$] scenario (10 times more sodium than regular concentrations) which enhances PTX effect.

As the potassium concentration increases, an event opposite to the one discussed the previous section is observed. In a High [K$^+$] scenario, the expected reward associated with PTXE changes to respectively 29.2241, 27.74% of the time. Therefore, as we increased [K$^+$], the likelihood of the pump to be bound exclusively to PTX decreased 23.17%. This result can be seen in Figures 6 and 7.

This result suggests that potassium inhibits PTX action. Therefore individuals with diets low in potassium, or with a pathology which decreases the potassium concentration in their metabolism could be more vulnerable to PTX. Potassium concentration could be increased to fight PTX action. In a similar way to sodium, there is another fine line here since a maximum amount of potassium is tolerated for one individual. There are a number of causes associated with a high potassium concentration (hyperkaulemia), such as renal insufficiency, Addison's disease, Gordon's syndrome and Rhabdomyolysis. Both results have been obtained from a parametric study of the state and transitions rewards of our model.

6.4   Species Depletion

We have also investigated properties related to species (ion or molecule) depletion events, i.e. there is no species in one side of the cell. For example, the
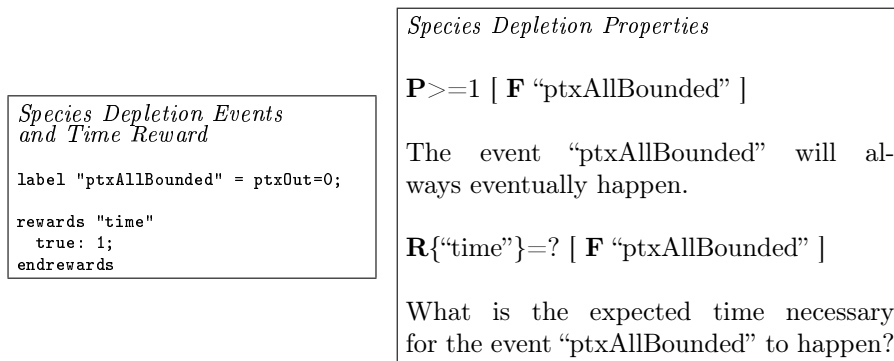
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Species Depletion Events and Time Reward

```
label "ptxAllBounded" = ptxOut=0;

rewards "time"
  true: 1;
endrewards
```

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Species Depletion Properties

P>=1 [**F** "ptxAllBounded"]

The event "ptxAllBounded" will always eventually happen.

R{"time"}=? [**F** "ptxAllBounded"]

What is the expected time necessary for the event "ptxAllBounded" to happen?

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Fig. 8. Depletion Events and Properties, and Time Reward

event "naOutDepletion" where there is no external sodium, or the event "ptxAll-Bounded" where all palytoxin molecules are bound to the pump. These events can be created in PRISM using *labels* (Figure 8).

Species depletion properties state that these events eventually (**F** operator) will always happen (**P**>=1 operator). For example, in every scenario the event "ptxAllBounded" eventually always happens. That is not the case for the event "naOutDepletion", which in every scenario it is not guaranteed that it will happen.

The event "kInDepletion" is sensitive to the parameter $[K^+]$– in the Control scenario, its property is true, while in the High $[K^+]$ scenario, the property becomes false, because it is more difficult to deplete internal potassium since there is 10 times more potassium. One could check how long it takes for those events to happen. For that we have to use a time reward, and reward properties, such as the one shown in Figure 8. The event "ptxAllBounded" is expected to happen in 1.7513E-5 seconds.

6.5 A Probabilistic and Quantified Kinetic Model

The classical Albers-Post model for the Na^+/K^+-ATPase was first proposed in [19]. It is a kinetic model which describes a set of directed chemical reactions that go from one state to another, consuming or producing substrates. We are able to quantify this kinetic model through PMC using state and transition rewards. We calculate a state probability dividing its reward by the sum of all state rewards. This is also applied to reactions and could be applied to substrates too.

We associate colors to states and reactions, in order to represent their probability. The kinetic model is colored using a jet palette which is often associated with temperatures, where probabilities transit from red to blue, or from likely to unlikely. This modified kinetic model is called a heat map. Red states and reactions are more probable or hot while blue states and reactions are unlikely or cold. An example of the heat map can be seen in Figure 9, where the states
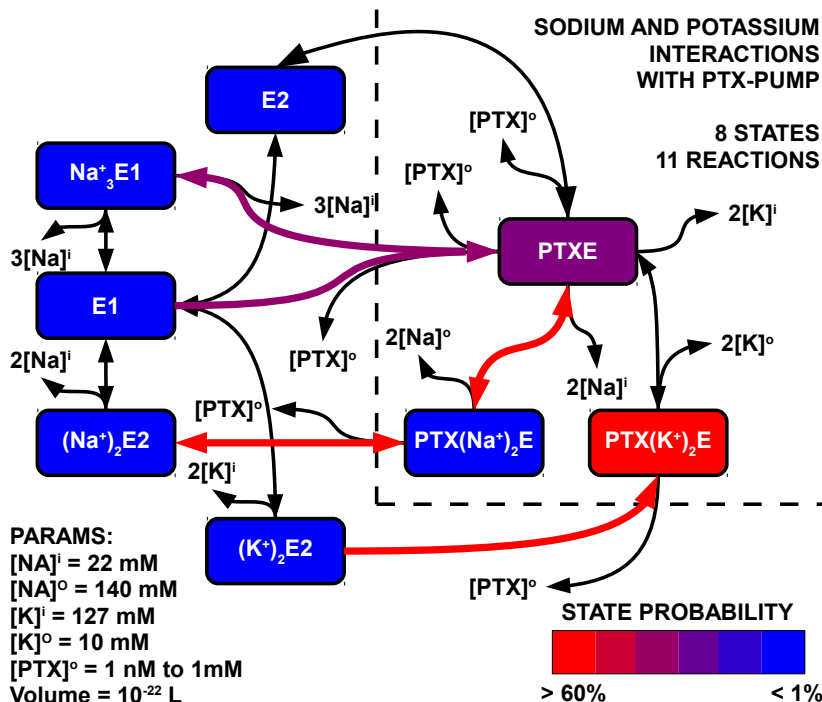
Fig. 9. Heat Map: kinetic model for the Na^+/K^+-ATPase with state and rate probabilities represented as colors. Each state and rate is colored based on its probability. Red states/rates are likely while blue states/rates are improbable. This could be a valuable tool for biologists as it shows model dynamics and it could be used to suggest overlooked experiments.

PTXK2E and PTXE are more probable, and reactions involving PTXNA2E occur more often.

The heat map could be a valuable tool for biologists as it shows model dynamics and it could be used to suggest overlooked experiments. Since the kinetic model is an abstraction suggested by experimental data, it could be incomplete, which the heat map would assist towards its completion. The heat map raises several questions, especially about likely reactions involved with improbable states. For example, the reaction between the states NA2E2 and PTXNA2E is one of the most actives, while the states themselves are the most inactives. This could suggest that there might be an intermediary state between these two states.

7   Conclusions and Further Work

The sodium-potassium pump $(Na^+/K^+$-ATPase) is a cellular structure which exchanges ions across the cell membrane. Its regular behavior is critical for

all animal cells, otherwise the individual could present some diseases or syndromes. A stochastic model representing the Na^+/K^+-ATPase has been built for a single pump using the Probabilistic Model Checking tool PRISM. In this model, the pump has been exposed to the toxin palytoxin (PTX), which drastically changes the pump regular behavior. PMC has allowed us to investigate the model, which show unpredictable and complex characteristics. Properties about biological events were expressed in probabilistic logics, e.g. "What is the probability of being in PTX related states?", which allowed the observation of rare events.

The results presented by the model have shown that high concentrations of sodium could enhance PTX effects. For example, when the sodium concentration is increased by 10 times its normal values, the probability of PTX inhibiting the pump increases 17,46%. This suggests that electrolyte disturbances could make an individual more susceptible to the toxin. Since PTX is found in an environment with a high concentration of sodium, this could represent some kind of evolutionary pressure.

An opposite behavior is observed regarding high concentrations of potassium. When potassium concentration is increased by 10 times its normal values, PTX effects are reduced by 23,17%. Both results suggest that electrolyte levels could be changed to reduce PTX effects on the pump by decreasing sodium and increasing potassium. Since electrolyte levels in the blood can be manipulated up to a certain degree, the study of their role and capability to change our Na^+/K^+-ATPase model behavior is even more important. PMC can improve our understanding of cell transport systems and its behavior, and can lead to the discovery and development of new drugs.

We have shown in this work that PMC can be used to obtain valuable information about cell transport systems in a simple and complete way. This type of analysis can provide a better understanding of how transmembrane ionic transport systems behave, helping in the discovery and development of drugs. Future work include performing electric current and ion concentration measurements; confront the results with experimental validation; explore other dimensions such as the number of pumps; and integrate to our model other toxins (e.g. ouabain) or drugs (e.g. digitalis).

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