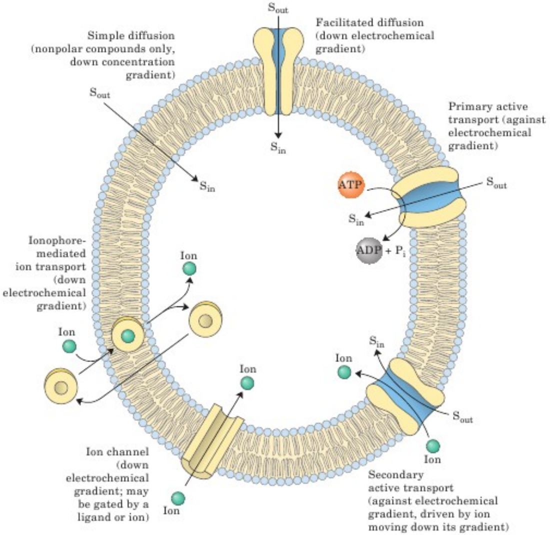
Analysis of Cell Membrane Ion Transport Systems using Model Checking

Sérgio Campos, Mirlaine Crepalde

Universidade Federal de Minas Gerais

July 2011

Cell Membrane Ion Transport Systems

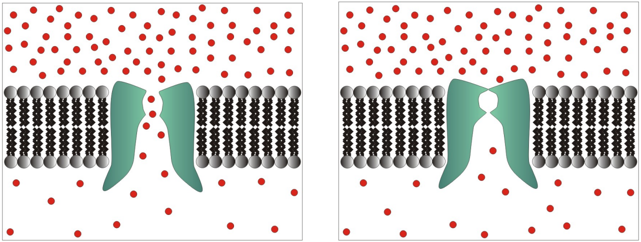


Ion Channels

- Fast passive flux of ions
- Animal toxin target
- Malfunction can cause serious illnesses

Defective Channel	*Pathology*
Sodium	Paralisia periódica hipercalêmica (Doença de Gamstrop) Paramiotonia congênita (Doença de Eulenburg) Miotonia atípica Síndrome do QT longo (gene LQT2)
Chloride	Fibrose cística Miotonia congênita (Doença de Thomsen) Miotonia generalizada (Doença de Becker)
Potassium	Síndrome do QT longo (genes LQT1 e LQT3)
Calcium	Paralisia periódica hipocalêmica Hipotermia maligna

Ion Channel Example

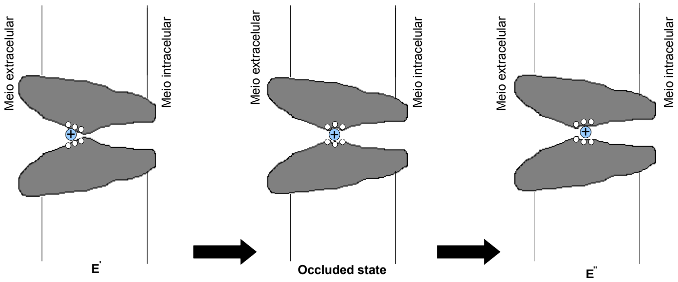


Open | Closed

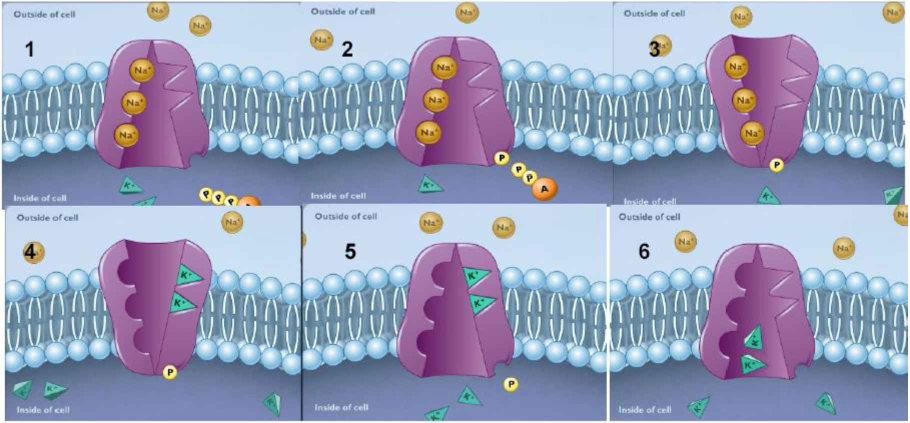
$$C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} O$$

Ion Pumps

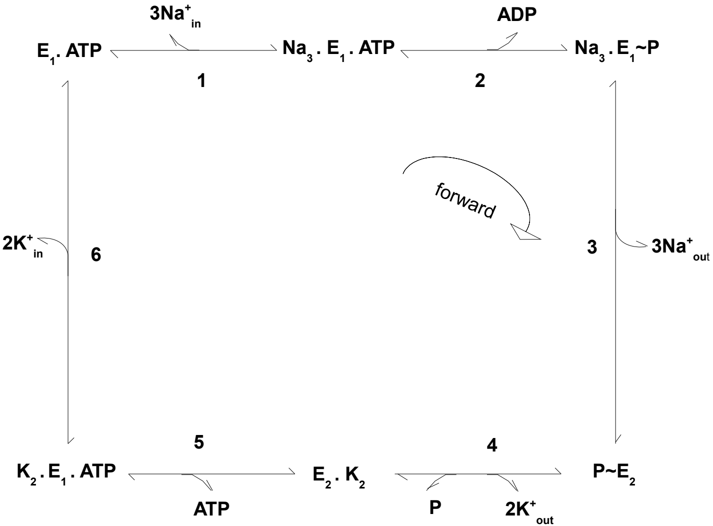
- Slow active flux of ions
- Animal toxin target
- Multiple states E_0, E_1, ..., E_n
- Cyclic reactions



The Sodium Potassium Pump



The Albers-Post Cycle



Model Parameters

Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
$[K^+_{in}]$	$0,12700$	M
$[K^+_{out}]$	$0,01000$	M
$[ATP]$	$0,00500$	M
$[P_i]$	$0,00495$	M
$[ADP]$	$0,00006$	M
f_1	$2,5 \times 10^{11}$	$M^{-3}s^{-1}$
f_2	10^4	s^{-1}
f_3	172	s^{-1}
f_4	$1,5 \times 10^7$	$M^{-2}s^{-1}$
f_5	2×10^6	$M^{-1}s^{-1}$
f_6	$1,15 \times 10^4$	s^{-1}
b_1	10^5	s^{-1}
b_2	10^5	$M^{-1}s^{-1}$
b_3	$1,72 \times 10^4$	$M^{-3}s^{-1}$
b_4	2×10^5	$M^{-1}s^{-1}$
b_5	30	s^{-1}
b_6	6×10^8	$M^{-2}s^{-1}$
cell volume	10^{-12}	l
temperature	310	K

Discreet Chemistry (counts ions and molecules)

- Discretizing concentrations
  - $\#X = [X] \times V \times N_{\mathrm{A}}$
- Discretizing rates
  - $r_i' = \frac{r_i}{(N_{\mathrm{A}} \times V)^{\kappa - 1}}$
  - $2A + B \rightharpoonup A_2B \; (\kappa = 3)$
- Law of mass action
  - $f_i = r_i' \times \prod_{i=j}^{n_i} \#X_j^{\kappa_{i,j}}$
  - $2A + B \rightharpoonup A_2B \; (\kappa_A = 2 \text{ e } \kappa_B = 1)$

```
module na
  naIn : [0..NI+NO] init NI;
  naOut : [0..NO+NI] init NO;
...
endmodule

module k
  kOut : [0..KO+KI] init KO;
  kIn : [0..KI+KO] init KI;
...
endmodule

module p
  p : [0..(Pi+ATPI+NP)] init Pi;
...
endmodule

module atp
  atp : [0..N] init ATPI;
...
endmodule
```

```
module adp
  adp : [0..(ADP+ATPI+NP)] init ADP;
...
endmodule

module pump
  E1ATP : [0..1] init 1;
  E1ATPNa : [0..1] init 0;
  E1PNa : [0..1] init 0;
  E2P : [0..1] init 0;
  E2K : [0..1] init 0;
  E1ATPK : [0..1] init 0;
...
endmodule

module base_rates
...
endmodule
```

$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \rightleftharpoons \mathrm{Na}_3.E_1.\mathrm{ATP}$$

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

  [r1] naIn>=naFlow -> pow(naIn,3) : (naIn'=naIn-naFlow);
  [rr1] naIn<=(NI+NO-naFlow) -> 1 : (naIn'=naIn+naFlow);
  ...
endmodule

module pump
  E1ATP : [0..1] init 1;
  E1ATPNa : [0..1] init 0;
  E1PNa : [0..1] init 0;
  E2P : [0..1] init 0;
  E2K : [0..1] init 0;
  E1ATPK : [0..1] init 0;

  //reaction1: 3 Na ions bind to pump enzyme
  [r1] E1ATP=1 & E1ATPNa=0 -> 1 : (E1ATP'=0) & (E1ATPNa'=1);
  [rr1] E1ATP=0 & E1ATPNa=1 -> 1 : (E1ATP'=1) & (E1ATPNa'=0);
  ...
endmodule

// module representing the base rates of reactions
module base_rates
  [r1] true -> r1rate : true;
  [rr1] true ->rr1rate : true;
...
endmodule
```

$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \underset{b_1}{\overset{f_1}{\rightleftharpoons}} \mathrm{Na_3}.E_1.\mathrm{ATP}$$

```
const double AV=6.022*pow(10.0,23);
const double V;

const int NI=ceil(0.022*AV*V);
const int NO=ceil(0.14*AV*V);
...

// base rates
const double r1rate = 2.5*pow(10,11)/(pow((V*AV),3));
const double rr1rate = 100000;
```

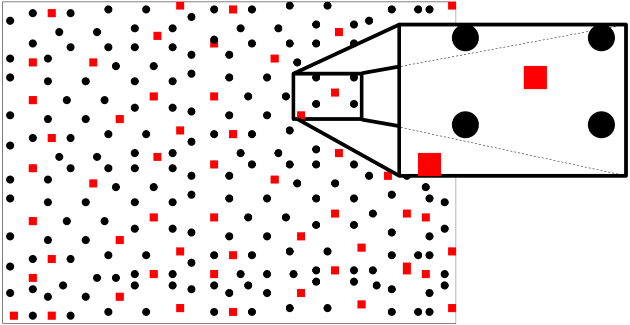
Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
f_1	$2,5 \times 10^{11}$	$M^{-3}s^{-1}$
b_1	10^5	s^{-1}

Variation of Cell Volume

Volume (l)	*# of states*	*N° transitions*	$Time_c\,(s)$	$Time_v\,(s)$
10^{-22}	9	16	0, 0318	0, 0010
10^{-21}	32	62	0, 3296	0, 0020
10^{-20}	194	386	48, 5324	0, 0050
10^{-19}	1838	3674	6745, 7930	0, 0460
10^{-18}	?	?	$>$ 7 dias	?

$$P_{\leq 0}\,[\,F\,(\,(atp = 0)\,\&\,!('\mathrm{naInOver}')\,\&\,!('\mathrm{kOutOver}')\,)\,]$$

Cell Volume Reduction



Individual Approach

```
module pump2=pump [
  E1ATP=E1ATP2,
  E1ATPNa=E1ATPNa2,
  E1PNa=E1PNa2,
  E2P=E2P2,
  E2K=E2K2,
  E1ATPK=E1ATPK2
]
endmodule

//system definition (Pumps do not interact with each other)
system
(pump ||| pump2) || na || k || p || adp || atp || base_rates
endsystem
```

Population Approach

```
...

const int NP;

...
module pump
  E1ATP : [0..NP] init NP;
  E1ATPNa : [0..NP] init 0;
  E1PNa : [0..NP] init 0;
  E2P : [0..NP] init 0;
  E2K : [0..NP] init 0;
  E1ATPK : [0..NP] init 0;

  //reaction1: 3 Na ions bind to pump enzyme
  [r1] E1ATP>0 & E1ATPNa<NP -> E1ATP : (E1ATP'=E1ATP-1) &

  [rr1] E1ATP<NP & E1ATPNa>0 -> E1ATPNa : (E1ATP'=E1ATP+1) &

        ...
endmodule
```

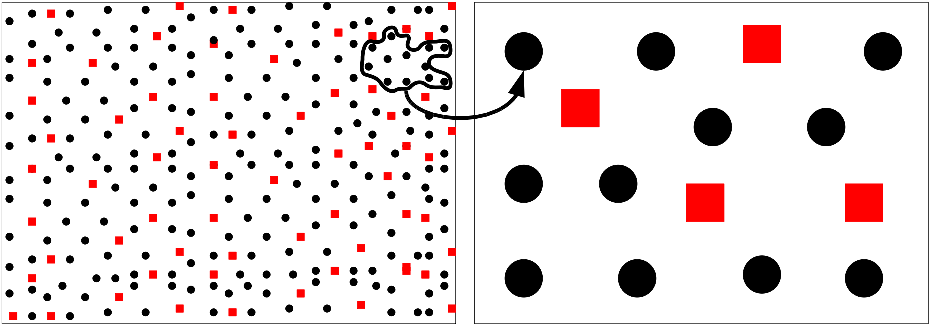
Population X Individual

NB	Population			Individual		
	Tamanho	T_c (s)	T_v (s)	*Tamanho*	T_c (s)	T_v (s)
1	194	49,6440	0,0050	194	47,0190	0,0050
2	686	63,0870	0,0100	1176	45,8160	0,0100
3	1848	51,4360	0,0240	7128	51,5630	0,0200
4	4200	87,4430	0,0390	43200	64,8940	0,0370
5	8484	100,7890	0,0710	261792	85,2880	0,0620
6	15708	137,9450	0,0930	$\approx 1,6 \times 10^6$	120,3400	0,0990
7	27192	153,5740	0,1630	$\approx 9,6 \times 10^6$	170,8320	0,1670
8	44616	284,4660	0,2480	$\approx 5,8 \times 10^7$	321,6320	0,3180
9	70070	449,5130	0,3810	$\approx 3,5 \times 10^8$	575,1240	0,4200
10	106106	783,4790	0,5310	$\approx 2,1 \times 10^9$	1047,9040	0,5190

Level Based Approach

- Variables describing substrate levels
  - Level 0 (no specimen present) till the maximum N_X
  - Distance from one level to the next is the size of the step h
- Concentration calculation
  - $[X] = l_{\mathrm{X}} \times h$
- Rate changes
  - $r_i'' = \frac{r_i}{h}$
- Law of mass action
  - $f_i = r_i'' \times \prod_{i=j}^{n_i} [X_j]^{\kappa_{i,j}}$

Level Based Approach



$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \rightleftharpoons \mathrm{Na}_3.E_1.\mathrm{ATP}$$

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

  [r1] naIn>=naFlow -> pow(naIn*h,naFlow) : (naIn'=naIn-naFlow) ;
  [rr1] naIn<=(NI+NO-naFlow) -> 1 : (naIn'=naIn+naFlow);
  ...
endmodule

module pump
  E1ATP : [0..NP] init NP;
  E1ATPNa : [0..NP] init 0;
  E1PNa : [0..NP] init 0;
  E2P : [0..NP] init 0;
  E2K : [0..NP] init 0;
  E1ATPK : [0..NP] init 0;

  //reaction1: 3 Na ions bind to pump
  [r1] E1ATP>0 & E1ATPNa<NP -> E1ATP*h : (E1ATP'=E1ATP-1) & (E1ATPNa'=E1ATPNa+1);
  [rr1] E1ATP<NP & E1ATPNa>0 -> E1ATPNa*h : (E1ATP'=E1ATP+1) & (E1ATPNa'=E1ATPNa-1);
  ...
endmodule

// module representing the base rates of reactions
module base_rates
  [r1] true -> r1rate : true;
  [rr1] true ->rr1rate : true;
...
endmodule
```

$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \underset{b_1}{\overset{f_1}{\rightleftharpoons}} \mathrm{Na}_3.E_1.\mathrm{ATP}$$

```
const double h;

const int NI=ceil(0.022/h);
const int NO=ceil(0.140/h);
...

// base rates
const double r1rate = 2.5*pow(10.0,11)/h;
const double rr1rate = 100000/h;
```

Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
f_1	$2,5 \times 10^{11}$	$M^{-3}s^{-1}$
b_1	10^5	s^{-1}

Step Size Variation

h	$\#$ *of states*	$\#$ *transitions*	$Time_c\ (s)$	$Time_v\ (s)$
0,0005	74	141	4,7238	0,0020
0,0004	86	172	4,5160	0,0020
0,0003	116	230	13,3520	0,0030
0,0002	164	326	30,0054	0,0050
0,0001	314	626	162,1990	0,0080
0,00009	350	698	202,1918	0,0080
0,00008	386	770	246,5536	0,0090
0,00007	440	878	361,3644	0,0140
0,00006	512	1022	510,0978	0,0130
0,00005	620	1238	648,9052	0,0190

BIOLAB Model

- Discretizing concentrations
  - $\#X = [X] \times V \times N_{\mathrm{A}}$
- Discretizing rates
  - $r_i' = \frac{r_i}{(N_{\mathrm{A}} V)^{\kappa-1}}$
  - $2A + B \rightharpoonup A_2B \ (\kappa = 3)$
- Law of Mass Action
  - Automatically incorporated to BIONETGEN

$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \underset{b_1}{\overset{f_1}{\rightleftharpoons}} \mathrm{Na}_3.E_1.\mathrm{ATP}$$

```
begin parameters

Na    6.02214179e23 #avogadro constant
V     1e-20         #cell volume
DIV   Na*V

# concentrations
sNaI  0.022*DIV #initial amount of Na inside cell
sNaO  0.14*DIV  #initial amount of Na outside cell
spump 1         #number of pumps
...

# rate constants
sT1   2.5e11 / DIV^3
sR1   100000
...

end parameters
```

Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
f_1	$2,5 \times 10^{11}$	$\mathrm{M}^{-3}\mathrm{s}^{-1}$
b_1	10^5	s^{-1}

```
begin molecule types
  pump(f~A~V,a,n1,n2,n3,k1,k2)
  Na(s~I~O~C,n)
  ...
end molecule types

begin seed species
  pump(f~A,a!1,n1,n2,n3,k1,k2).A(u!1,p1!2,p2!3,p3!4).P(p!2).P(p!3).P(p!4) spump
  Na(s~I,n)                    sNaI
  Na(s~O,n)                    sNaO
  ...
end seed species
```

$$E_1.\mathrm{ATP} + 3\mathrm{Na}^+_{\mathrm{in}} \underset{b_1}{\overset{f_1}{\rightleftharpoons}} \mathrm{Na}_3.E_1.\mathrm{ATP}$$

```
begin reaction rules

pump(f~A,a!1,n1,n2,n3,k1,k2).A(u!1,p1!2,p2!3,p3!4).P(p!2).P(p!3).P(p!4) +
Na(s~I,n) + Na(s~I,n) + Na(s~I,n) <->
pump(f~A,a!1,n1!5,n2!6,n3!7,k1,k2).A(u!1,p1!2,p2!3,p3!4).P(p!2).P(p!3).P(p!4).
Na(s~C,n!5).Na(s~C,n!6).Na(s~C,n!7) sT1,sR1

...
end reaction rules
```

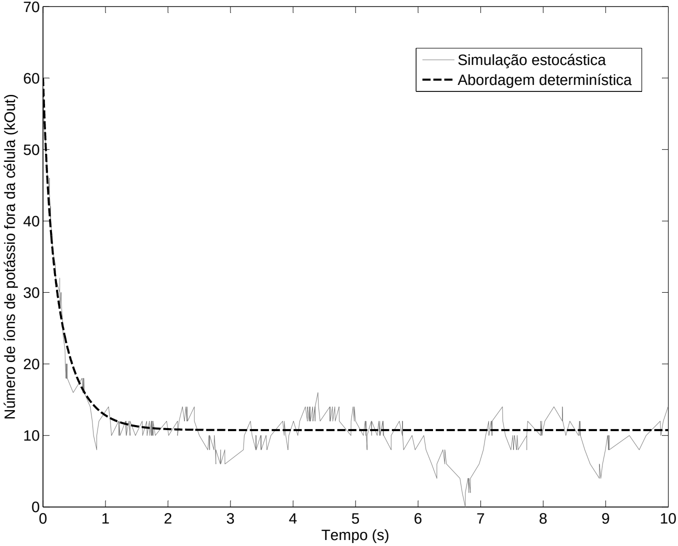
Cell Volume Variation

Tabela: $t_{end} = 10$, $n_{steps} = 1000$, $\alpha = 0,01$ e $\beta = 0,01$.

Volume (l)	$Time_v(s)$
10^{-22}	36,7500
10^{-21}	36,6440
10^{-20}	35,9650
10^{-19}	36,3350
10^{-18}	36,0020
10^{-12}	36,1240

Variaton of the Number of Pumps

NP	$Time_v(s)$
1	35,9650
2	38,1660
4	42,9770
6	48,0260
8	53,5290
10	58,8940
100	357,5220
1000	1734,1940



Simulação estocástica
Abordagem determinística
Número de íons de potássio fora da célula (kOut)
Tempo (s)
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6
7
8
9
10

Properties Verified

- $P \geq 1\,[\,F \quad \text{'kOutOver'}\,]$: potassium outside the cell will always end
- $R\{\text{'time'}\} =?\,[\,F \quad \text{'kOutOver'}\,]$: Expected time for potassium to end is 1287 seconds
- $P =?\,[\,F \leq 1287 \quad \text{'kOutOver'}\,]$: in 63% of the paths the potassium ends until 1287 seconds
- $P =?\,[\,F \leq 10 \quad \text{'kOutOver'}\,]$: in 0.63% of the paths the potassium ends in less than 10 segundos

Properties Verified

- $P \geq 1\,[\,G \quad (\text{'kOutOver'} \Rightarrow P \geq 1\,[\,F \quad \text{kOut} \geq \text{KO}\,]\,)\,]$: if the potassium ends outside the cell, will it always return to its original state?

- $R\{\text{'time'}\} =?\,[F \quad \text{kOut}{=}\text{KO} \quad \{\text{'kOutOver'}\}]$: time expected for it to return is 132.515 seconds

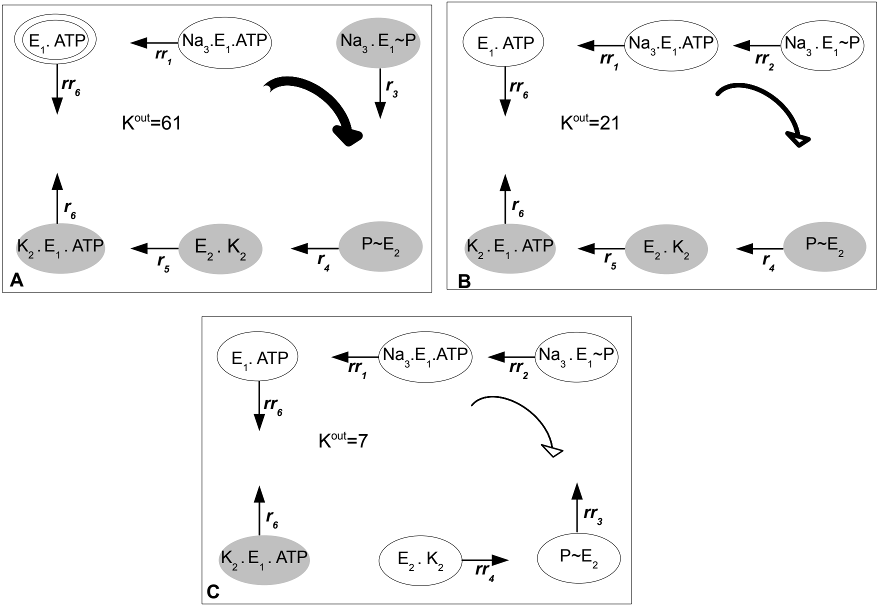
Pump Reversibility

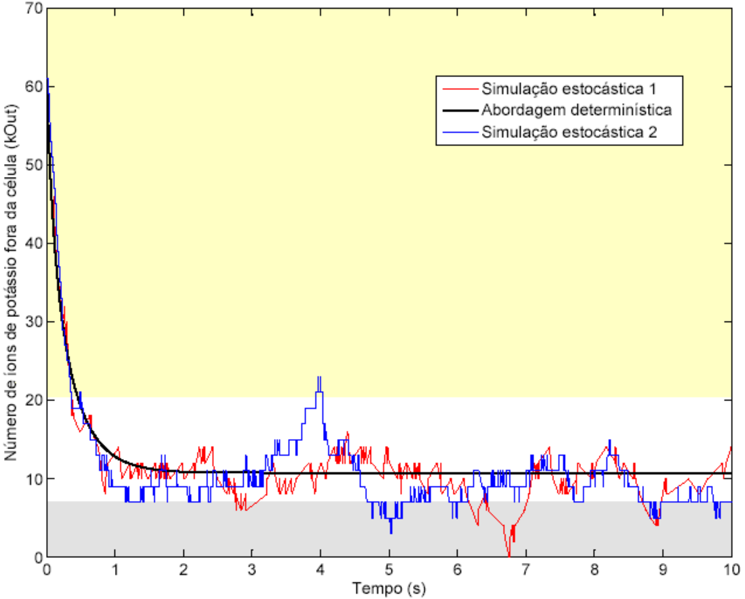
- Potassium outside the cell reaches maximum and mininimum values indefinitely.

$$P \geq 1\,[G\,((\text{kOut}{=}\text{KO} \Rightarrow (P > 0\,[F \quad \text{'kOutOver'}]))| \\ (\text{'kOutOver'} \Rightarrow (P > 0\,[F \quad \text{kOut}{=}\text{KO}])))] \tag{1}$$

Study of Tendencies — First Study

Parâmetro	*Valor*	*Unidade*
$[Na_{in}^{+}]$	$0,02200$	M
$[Na_{out}^{+}]$	$0,14000$	M
$[K_{in}^{+}]$	$0,12700$	M
$[K_{out}^{+}]$	$0,01000$	M
$[ATP]$	$0,00500$	M
$[P_i]$	$0,00495$	M
$[ADP]$	$0,00006$	M


E1. ATP
rr1
Na3.E1.ATP
Na3 . E1~P
rr6
r3
Kout=61
r6
K2 . E1 . ATP
r5
E2 . K2
r4
P~E2
A
E1. ATP
rr1
Na3.E1.ATP
rr2
Na3 . E1~P
rr6
Kout=21
r6
K2 . E1 . ATP
r5
E2 . K2
r4
P~E2
B
E1. ATP
rr1
Na3.E1.ATP
rr2
Na3 . E1~P
rr6
Kout=7
r6
rr3
K2 . E1 . ATP
E2 . K2
rr4
P~E2
C

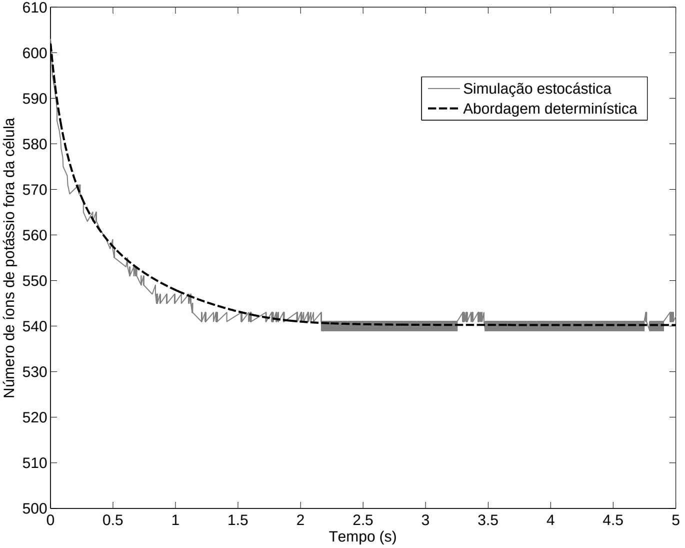


Simulação estocástica 1
Abordagem determinística
Simulação estocástica 2
Número de íons de potássio fora da célula (kOut)
Tempo (s)
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6
7
8
9
10

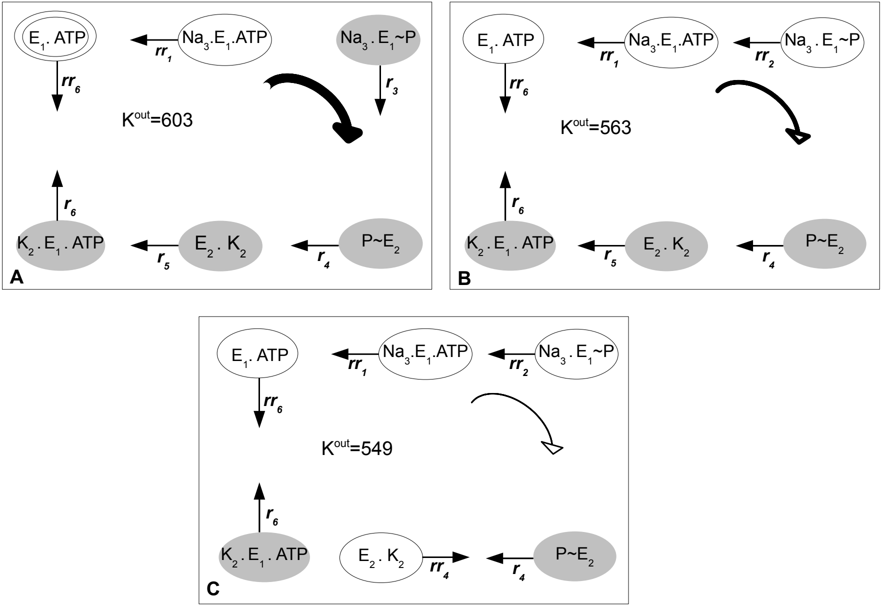
Second Study — Increasing Potassium Concentration Outside the Cell

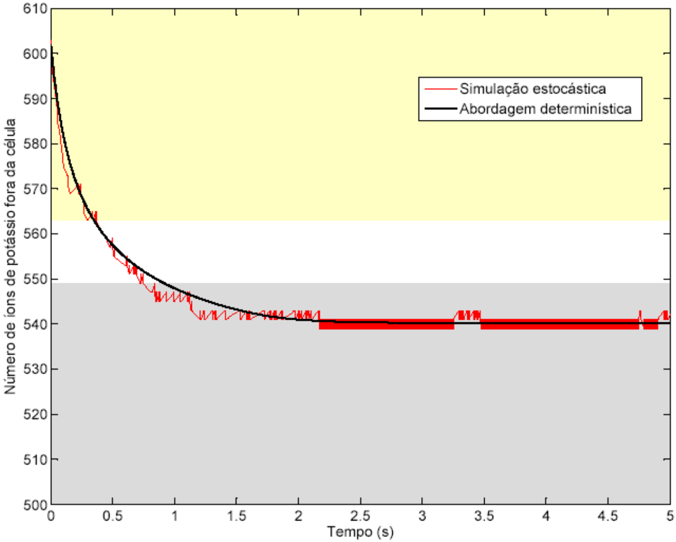
Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
$[K^+_{in}]$	$0,12700$	M
$[K^+_{out}]$	$0,01000$	M
$[ATP]$	$0,00500$	M
$[P_i]$	$0,00495$	M
$[ADP]$	$0,00006$	M

Parameter	*Value*	*Unit*
$[Na^+_{in}]$	$0,02200$	M
$[Na^+_{out}]$	$0,14000$	M
$[K^+_{in}]$	$0,12700$	M
$[K^+_{out}]$	$0,10000$	M
$[ATP]$	$0,00500$	M
$[P_i]$	$0,00495$	M
$[ADP]$	$0,00006$	M



Simulação estocástica
Abordagem determinística
Número de íons de potássio fora da célula
Tempo (s)
610
600
590
580
570
560
550
540
530
520
510
500
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5


E1. ATP
Na3.E1.ATP
Na3 . E1~P
rr1
rr6
r3
Kout=603
r6
K2 . E1 . ATP
E2 . K2
P~E2
r5
r4
A
E1. ATP
Na3.E1.ATP
Na3 . E1~P
rr1
rr2
rr6
Kout=563
r6
K2 . E1 . ATP
E2 . K2
P~E2
r5
r4
B
E1. ATP
Na3.E1.ATP
Na3 . E1~P
rr1
rr2
rr6
Kout=549
r6
K2 . E1 . ATP
E2 . K2
P~E2
rr4
r4
C



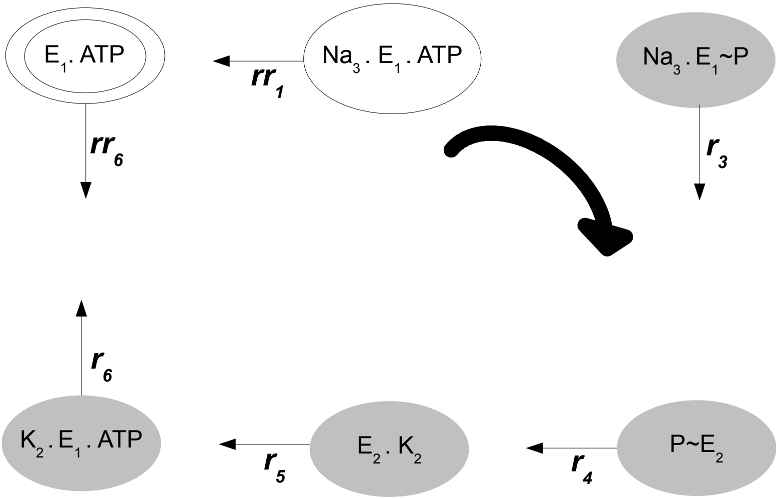
Simulação estocástica
Abordagem determinística
Número de íons de potássio fora da célula
Tempo (s)
610
600
590
580
570
560
550
540
530
520
510
500
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

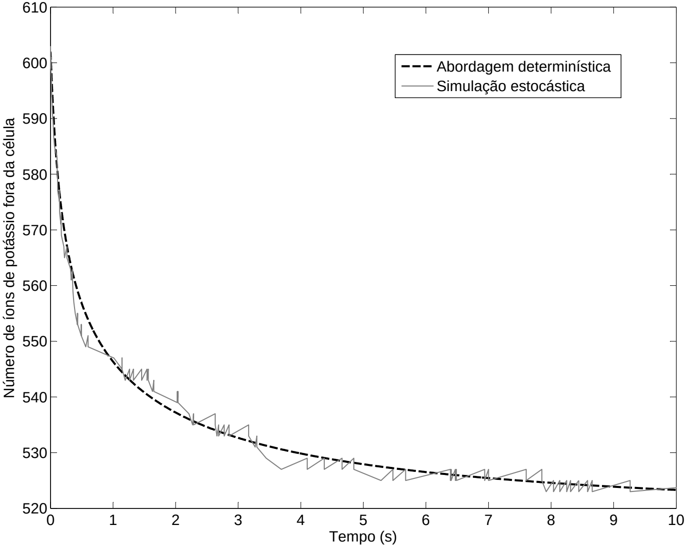
Third Study — ATP Synthesis

- Oxidative Phosporylation inside the cell

- $\mathrm{ADP} + \mathrm{P_i} \overset{rATP}{\rightarrow} \mathrm{ATP}$

```
module p
        ...
        [rATP] p>0 -> p : (p'=p-1);

endmodule

module atp
  ...
        [rATP] atp<N -> 1 : (atp'=atp+1);

endmodule

module adp
  ...
        [rATP] adp>0 -> adp : (adp'=adp-1);

endmodule

const double rATP = 1000.0/pow(V*AV,1);

module start
  ...
        [rATP] true -> rATP : true;

endmodule
```


E1. ATP
Na3 . E1 . ATP
Na3 . E1~P
rr1
rr6
r3
r6
K2 . E1 . ATP
E2 . K2
P~E2
r5
r4


Abordagem determinística
Simulação estocástica
Número de íons de potássio fora da célula
Tempo (s)
610
600
590
580
570
560
550
540
530
520
0
1
2
3
4
5
6
7
8
9
10

Pump is Interrupted

- $P \geq 1\,[\,F\,\text{'naInOver'}\,]$: sodium runs out outside the cell
- when outside potassium is 517

$$KO + 2 * ((R\{\text{'plusKout'}\} =?\,[F \quad \text{'naInOver'}\,])- (R\{\text{'minusKout'}\} =?\,[F \quad \text{'naInOver'}\,])) \tag{2}$$

Conclusions and Future Work

- Several techniques to model Na,K-ATPase
- Analysis of Na,K-ATPase
  - Absence of substrates
  - Pump reversibility
  - Tendencies study

Future work:

- Applying to other systems
- Experimental validation
- Introducing new variables, e.g. toxins