In Silico Laboratory Experiments using Statistical Model Checking: A new model of the Palytoxin-Induced Pump Channel as Case Study

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Understanding Biological Systems

Understanding biological systems is vital. And hard.

- Laboratory experiments:
  - Difficult
  - Expensive — money and time
- Simulation
  - Less expensive... Once you have the computing power...
  - Unable to guide simulations
- Our proposal: *Statistical Model Checking*
  - inexpensive — so far, experiments performed on notebooks.
  - Able to model system and guide experiments
  - Uncertainty known: Faster if you require less accuracy

Statistical Model Checking

Combines model checking and simulation:

- Simulation engine
- Temporal logic properties guide the analysis process
  - For example, introduction of a toxin at a certain time
- Validity of a property is estimated from a number of execution traces
- Confidence margin not achieved ? Repeat...

We use UPPAAL-SMC.

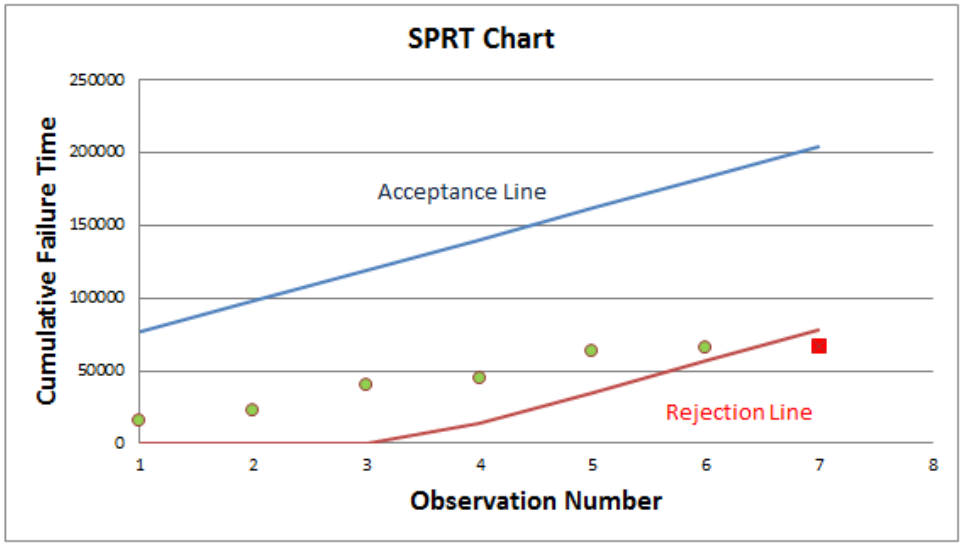
Statistical Model Checking

Each run consists of a series of steps:

- Choose a valid transition according to transition rates
- Update variables according to transition rules
- repeat

Once an execution run is finished, record: success/failure

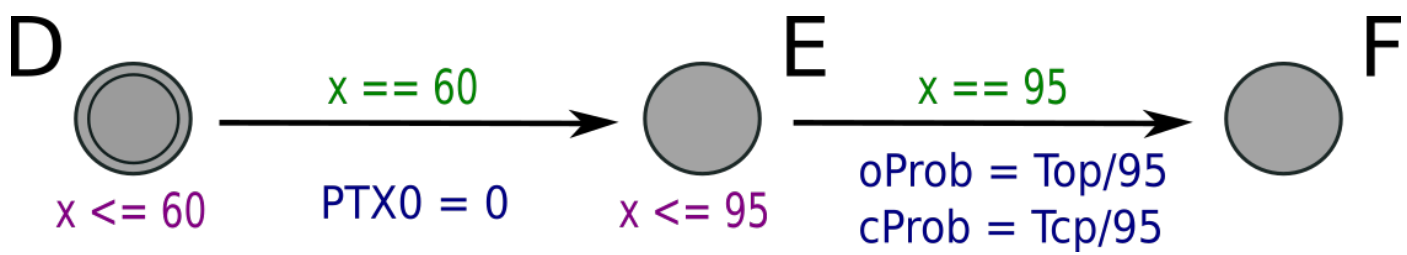
SPRT algorithm decides if confidence error has been reached.



SMC Model

A Priced timed automata (PTA) is a tuple $(S, S_0, X, \Sigma, E, R, I)$ where

- S is a set of states, S_0 a initial state;
- X is a finite set of clocks;
- $\Sigma = \Sigma_i \cup \Sigma_o$ is a finite set of actions separated into input and output actions;
- E is a set of transitions
- R and I are functions assigning rate vector and invariants to each state.



Properties

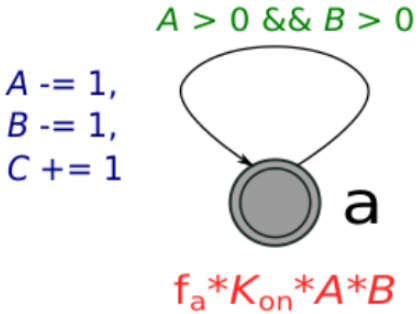
Properties can be expressed in:

- CTL: **AG**(toxin $\rightarrow$ **AF** death): Will the toxin cause death in the future ?
- WMTL: Weighted Metric Temporal Logic
  - $P[10]_{=?}$[toxin $\rightarrow$ **F** death] What is the probability that the toxin will cause death in ten seconds?
  - $P[10]_{>.9}$[toxin $\rightarrow$ **F** death] Is the probability of death in ten seconds after a toxin greater than 90% ?

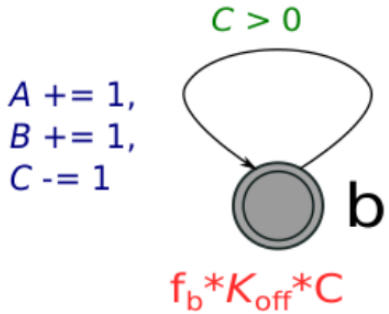
Translation Principles

$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C \xrightarrow{k_{deg}}$$

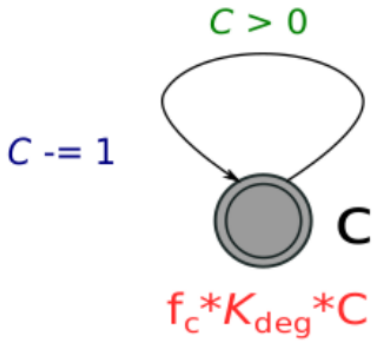
a: $A + B \rightarrow C$ $r(a) = K_{on}[A][B]$



b: $C \rightarrow A + B$ $r(b) = K_{off}[C]$



c: $C \rightarrow$ $r(c) = K_{deg}[C]$



Translation Principles

If we have

- $A = 50$, $B = 80$, $C = 0$,
- $K_{on} = 0.2e^{-1}$, $K_{off} = 0.1e^{-1}$, and $K_{deg} = 1.0e^{-1}$

we can ask things such as:

```
Pr[x<=60] (<> C >= 12 && C <= 18 && x > 30)
```

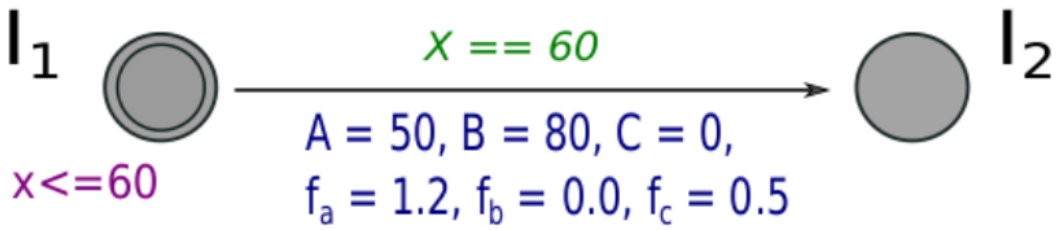
which returns

```
(36 runs) Pr(<> ..)  in [0,0.097393] with
confidence 0.95
```

Modeling Experimental Steps

We can do more. Say we want to restart the experiment:

- at 60 seconds;
- increasing production of C by 20%
- decreasing degradation by 50%.

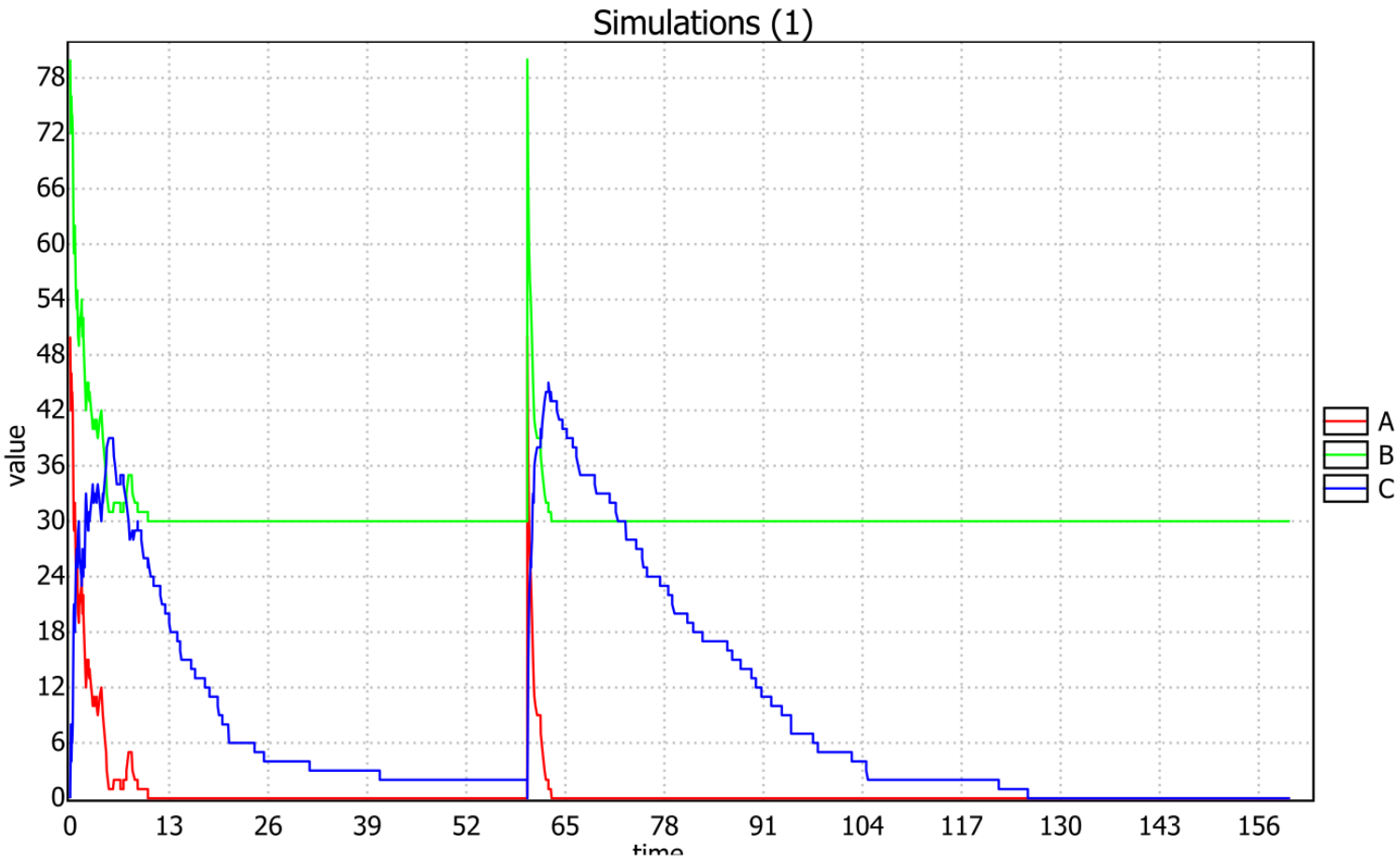



```
Pr[x<=120] (<> C >= 12 && C <= 18 && x > 90)
```

results in

```
(402 runs) Pr(<> ..)  in [0.46734,0.56721] with
confidence 0.95.
```

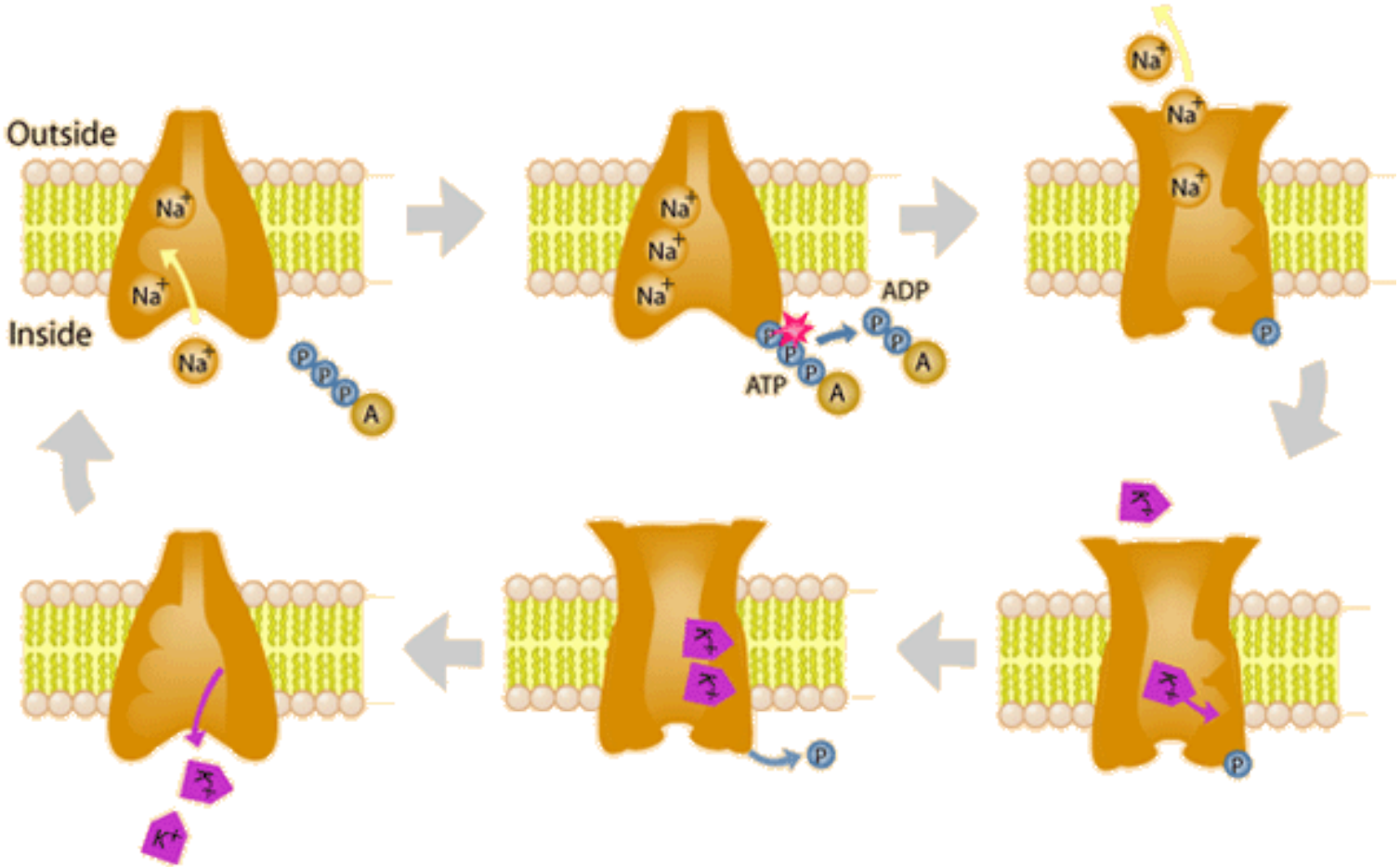
Modeling Experimental Steps



Case Study: The Na+/K+ Pump Complex

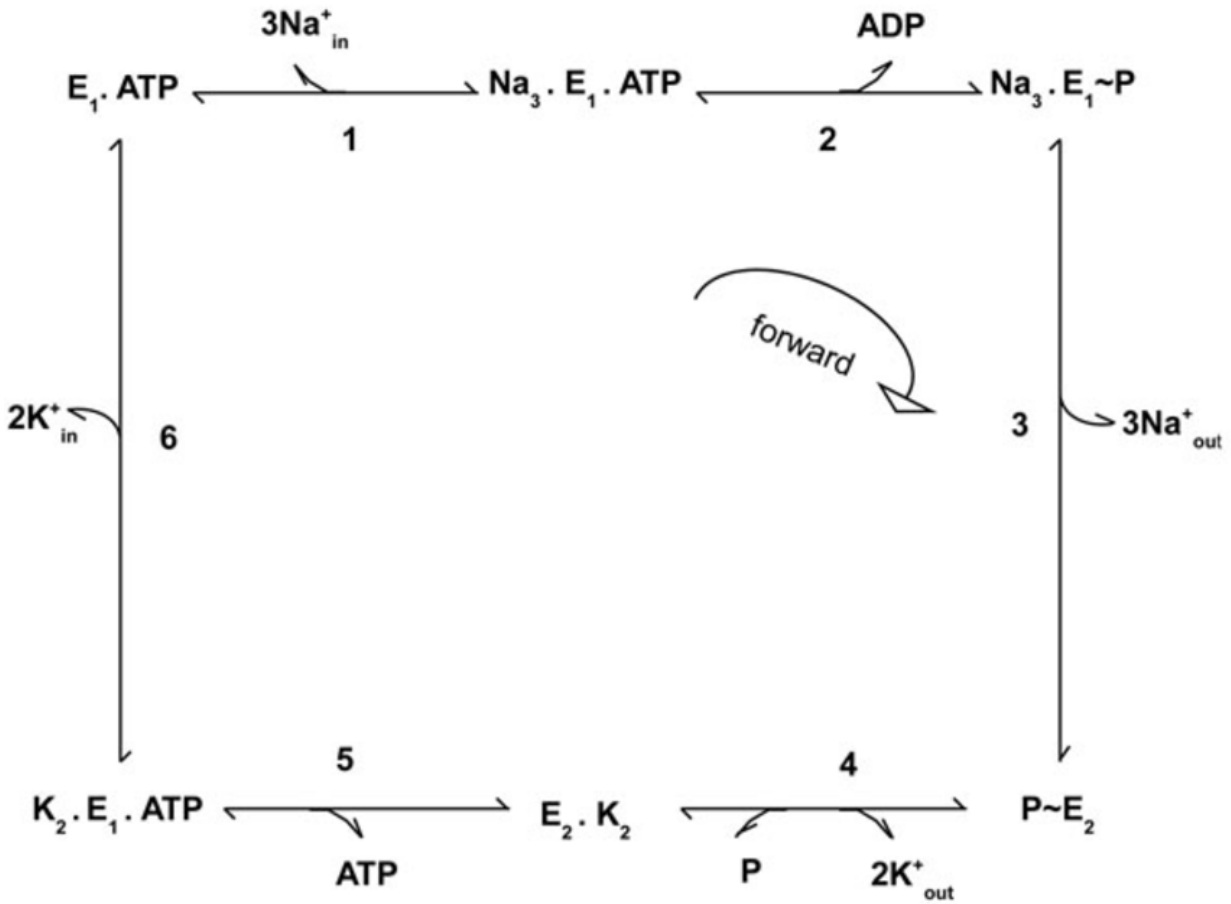
- Mechanism by which cells move sodium and potassium ions across the membrane.
- It is an active process and involves the hydrolysis of adenosine triphosphate (ATP).
- Crucial for cell physiology, the non-electrical communication mechanism between cells.
- Toxins can block pumps... And cause death

Case Study: The Na+/K+ Pump



Case Study: The Na+/K+ Pump

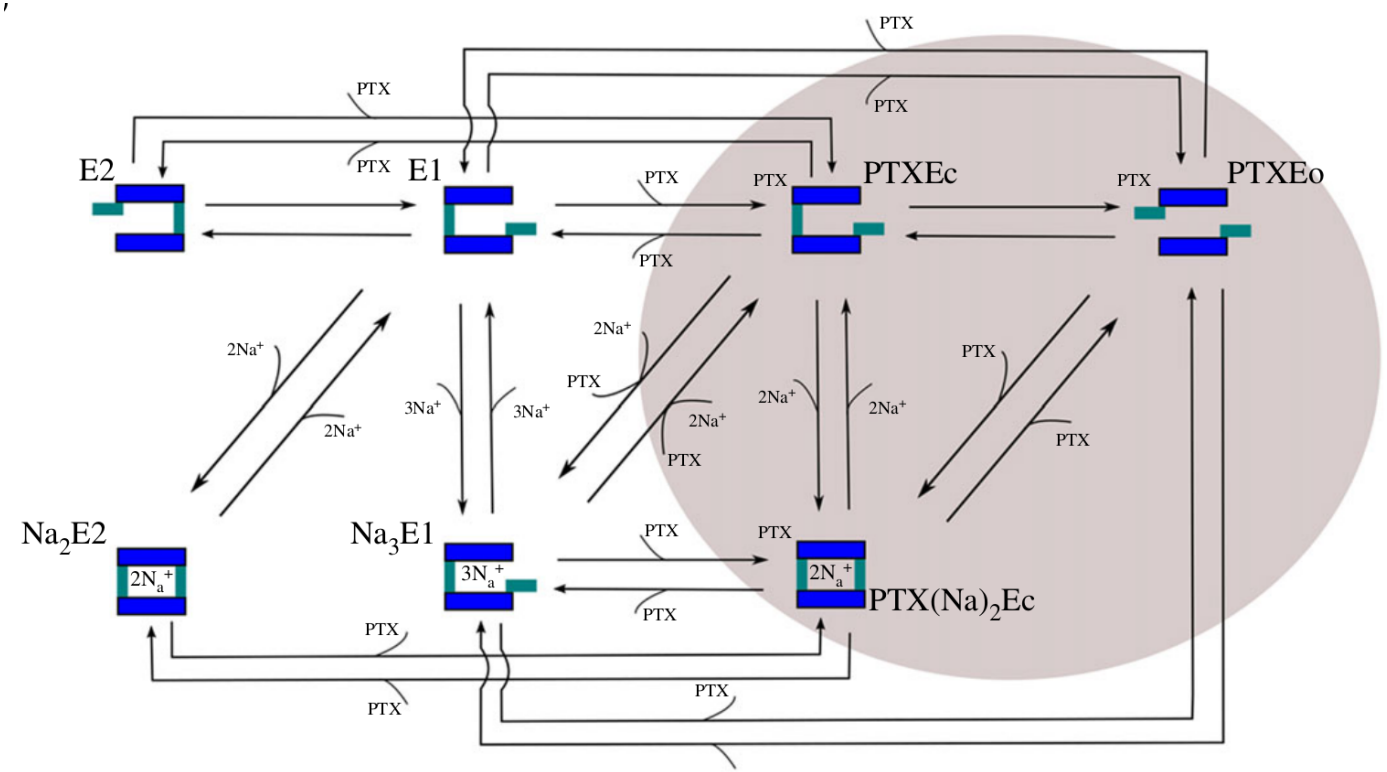
It works in a cycle, proposed by Albers and Post in 1965:



Case Study: The Na+/K+ Pump

We want to know how Palytoxin affects the pump complex.

- We created a whole-cell model based on an existing differential equations model
- State PTXE represents an active toxin
- When PTX is active, the pump is open 19% of the time.
- We then replaced PTXE with PTXEo and PTXEc.



First Model — Whole-Cell

The hard part:

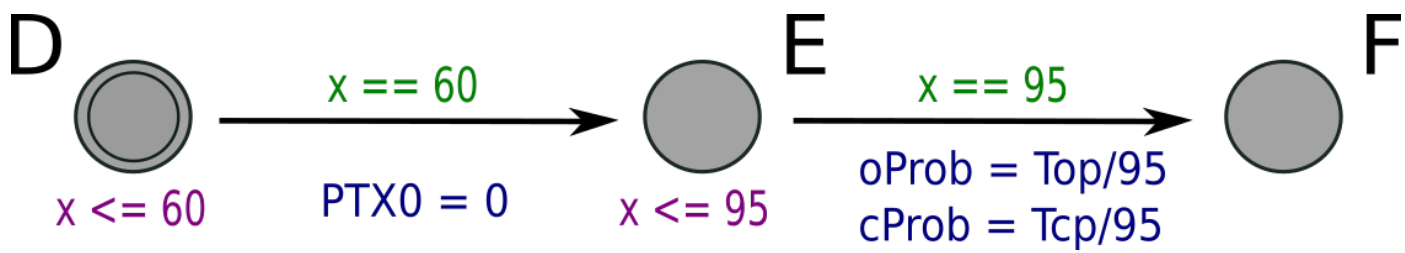
- Defined a new reaction $PTXEc \leftrightarrow PTXEo$ with $r_{p8} = \alpha_{p8}[PTXE_c] - \beta_{p8}[PTXE_o]$
- Determine α_{p8} and β_{p8} such that 19% of the time is spent in the PTXEo state.
- Initial conditions based on literature experiments:
  - $[Na^+]i = 150\text{nM}$, $[Na^+]o = 160\text{nM}$, $[PTX]o = 2.0\text{nM}$.
- Iterated simulations until *oProb* was 19%:
  - $\alpha_{p8} = 1.33$, $\beta_{p8} = 5.09$

First Model — Whole-Cell

We can now reproduce experiments:

- Initially, $[PTX]o = 2.0\text{nM}$.
- After 60s, PTX is removed
- Activity is tracked for another 35s (95s total)

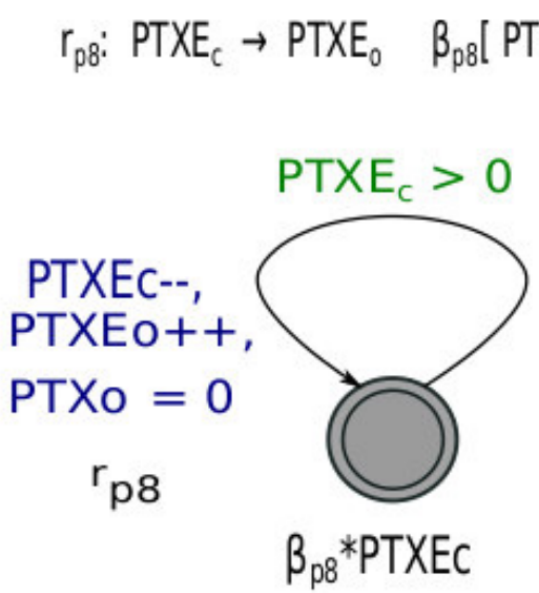
Behavior consistent with literature



Second Model — Single-Cell

Moving on... Another paper studied a single cell configuration

- Same initial condition: $[PTX]o = 2.0\text{nM}$.
- When the first channel is open, remove PTX.
- Induced channel stays active after PTX is removed.



Diprotomeric Configuration

Problem: different behaviors!!!
The whole-cell model with n-pumps set to 1 does not work.

The literature speculates that the pump might actually have *two binding* sites for PTX

- One with high susceptibility
- One with lower susceptibility

but no evidence for it has been found.

- Difficult to perform experiment

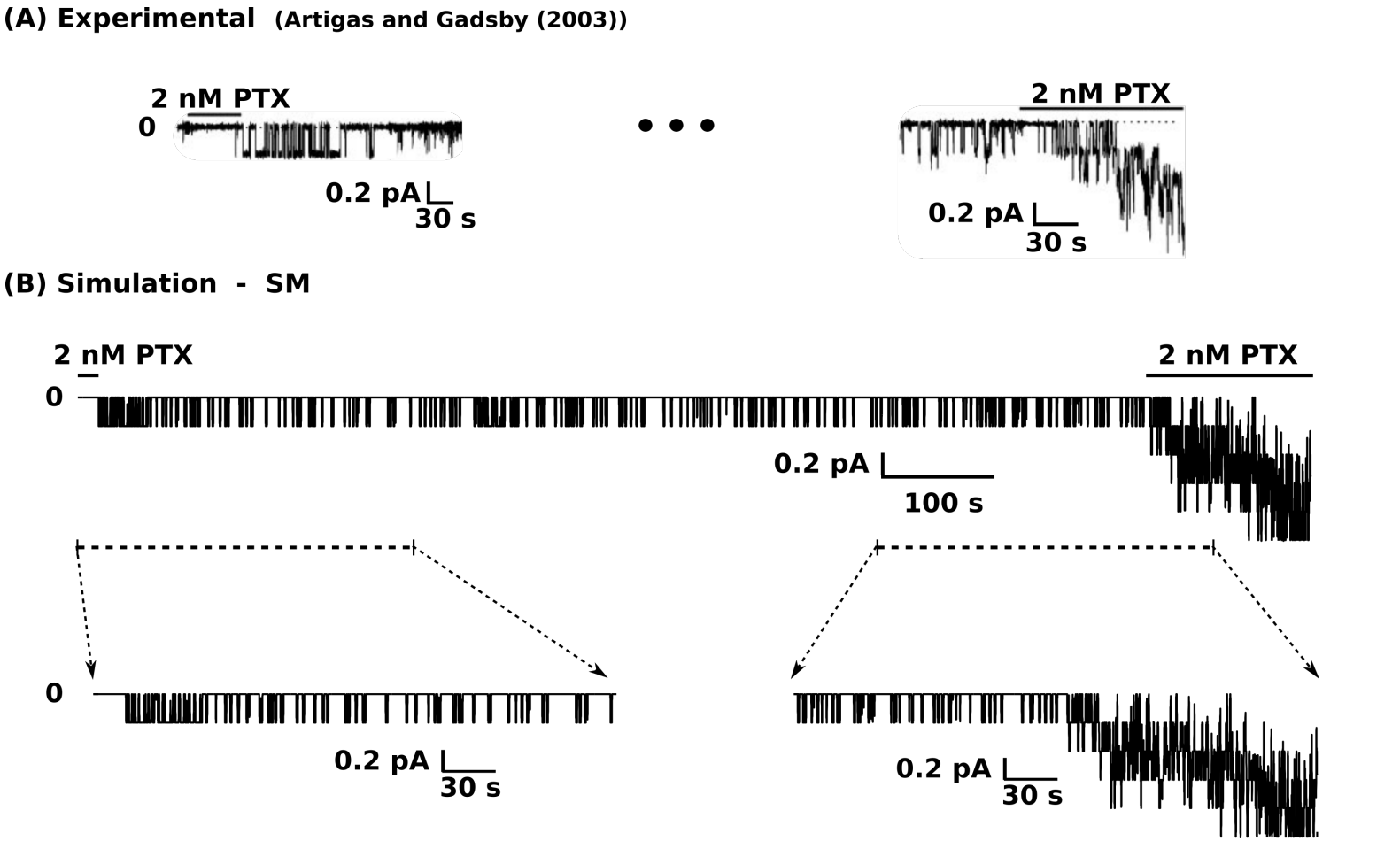
We modelled the diprotomeric model:

- Our model worked perfectly, for both configurations

Diprotomeric Configuration

We simulated it for 1100s:

- 60s until channel opens
- 12m until PTX is reintroduced



Conclusions

- SMC provided the first evidence of a theoretical model
- Obtained biological scientific results
- Very efficient:
  - Typically clusters are needed for simulation
  - We used only a regular notebook
  - Execution used a few MB, a few seconds