Stochastic Formal Model of PI3K/mTOR Pathway in Alzheimer's Disease: An Evaluation of Rapamycin, LY294002, and NVP-BEZ235

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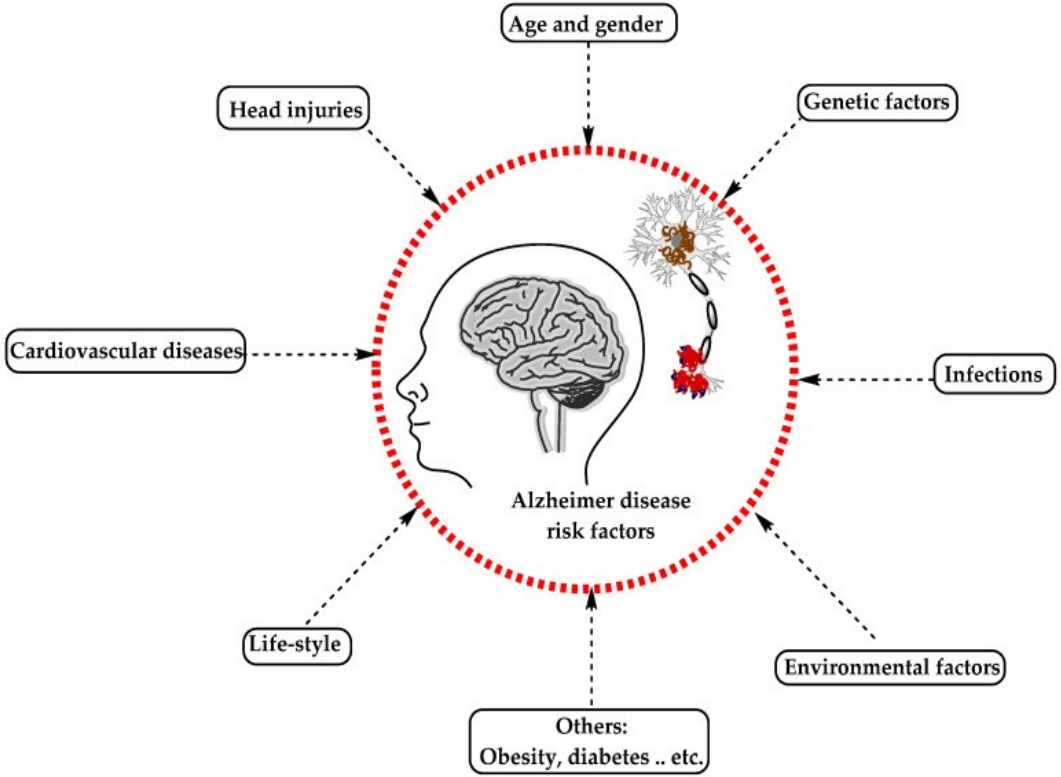
Laboratório de Universalização de Acesso

Alzheimer’s disease

- Alzheimer's disease is the most prevalent form of dementia.
- Memory loss is the best-known clinical condition.
    - Disorientation, difficulty communicating, depression, among others.
- The number of patients grows every year.
    - 3 of 5 people in the world will be diagnosed with the disease in 2050.

Alzheimer’s disease



Fonte: Breijyeh *et al.*, 2020

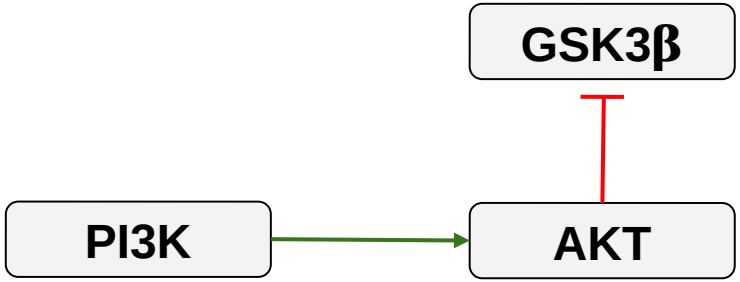
Drug Repurposing

- Drug repositioning and combination therapies are alternatives of treatments.
  - Reduced development time and investment (De castro *et al.*, 2018).

    Traditional drug development methods can take 10 to 17 years.

    Repositioning can take between 3 and 12 years.

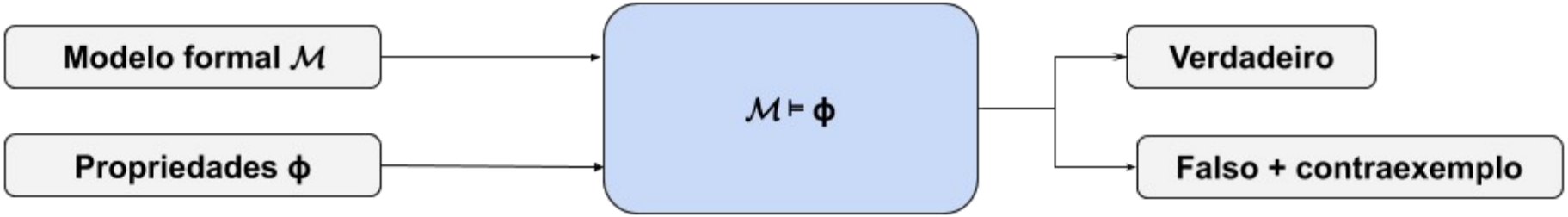
Biological Systems



- *In silico* techniques can contribute to this process.
  - Evaluation of side effects.
  - insights
- Models
  - They are abstractions of key components of a system.
- There are different computational techniques for analyzing models, such as simulation and automatic verification.

Model Checking

Model Checking is an automatic technique for exhaustive system verification.



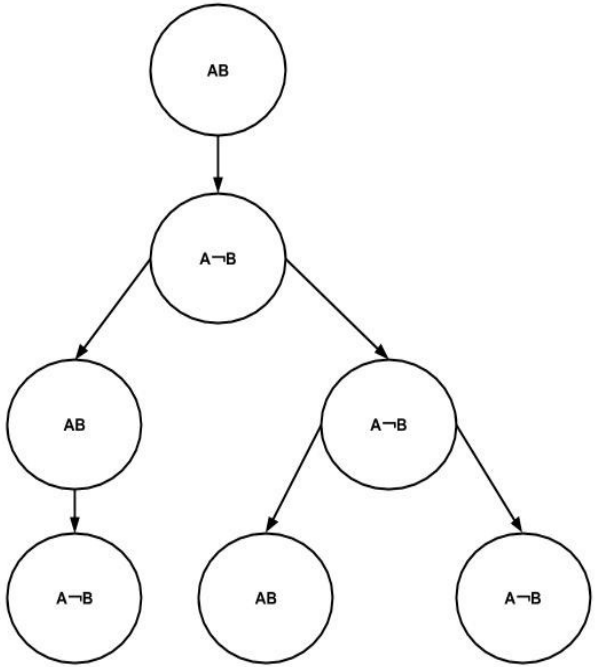
“Does mTORC1 inhibition imply an increase in autophagy?”

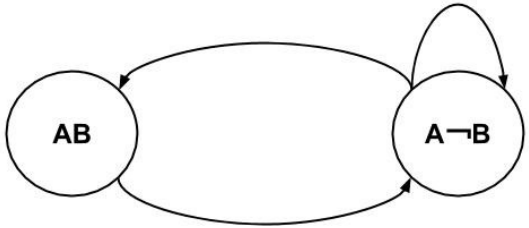
“Does PI3K inhibition imply an increase in Tau phosphorylation?”

Bottleneck

There is no free lunch

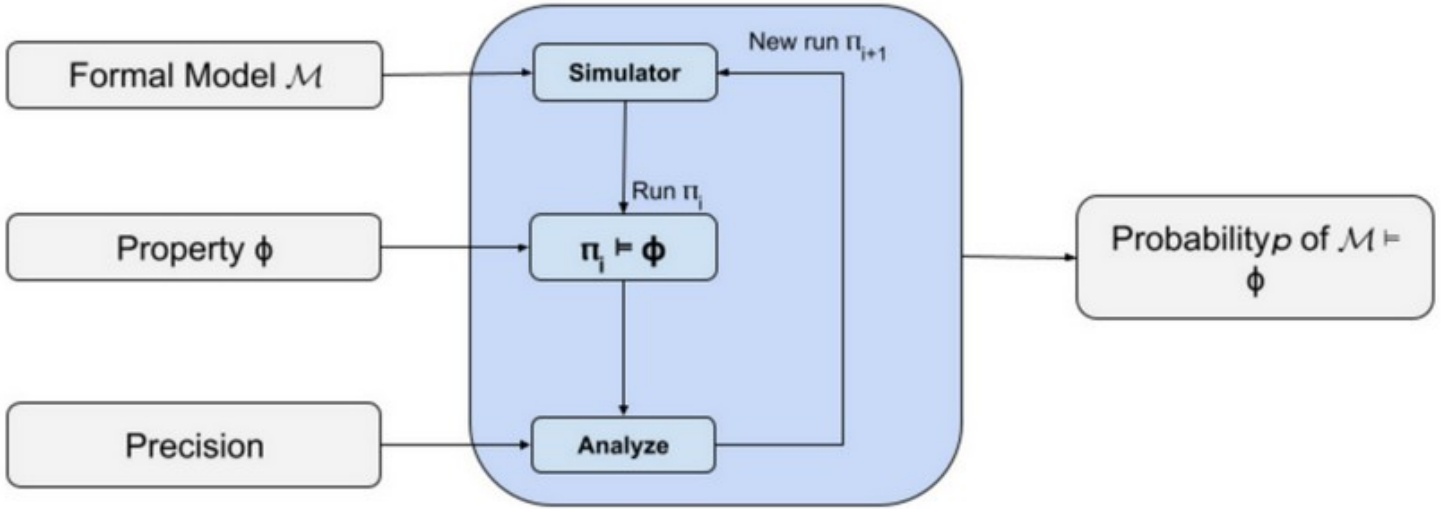
- In complex systems, state explosion occurs.
  - The number of states grows exponentially.





Statistical Model Checking (SMC)

SMC is a technique for automatic analysis of complex systems that verifies properties in a temporal logic, using controlled simulations.



Temporal Logic - WMTL

- **Qualitative**

Pr[<=86400*4](<> !controllNFT.produced) >= 0.7

"Hypothesis testing" to make sure a property is satisfied with a certain degree of probability.

- **Quantitative**

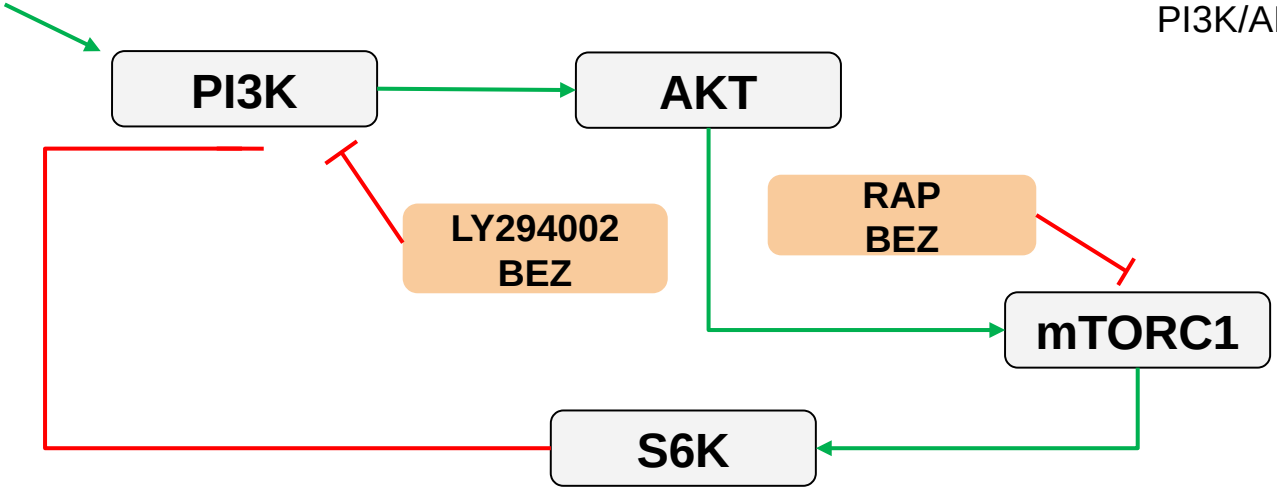
Pr[t<=86400*2]([] !controllAbetaPlaque.produced)

"Estimate" that allows you to identify the probability of a property being satisfied.

Goal

Apply SMC technique to verify the potential pharmacological effects in inhibiting the PI3K-Akt-mTOR pathway for the treatment of Alzheimer's disease.

PI3K/mTOR pathway



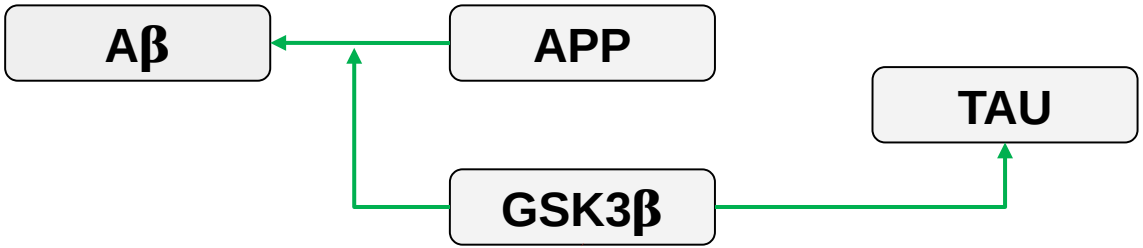
PI3K/AKT/mTOR pathway

Related work

Summary of mathematical models used as reference in this work. None of the models integrate the PI3K/mTOR pathway with Alzheimer's Disease nor the stochastic behavior.

Scope	Model	Variables	Model Type
PI3K/mTOR	[29]	IR-IRS1-Akt-TSC-mTORC1-S6K1	ODE
	[30]	IR-PI3K-Akt-mTOR-S6K-GSK3 β	ODE
	[27]	Rapamycin-mTOR	ODE
	[31]	IR-IRS-Akt-mTORC1-mTORC2-DEPTOR	ODE
	[32]	Rapamycin-mTORC1-mTORC2	ODE

Aβ/Tau pathway



Alzheimer’s disease

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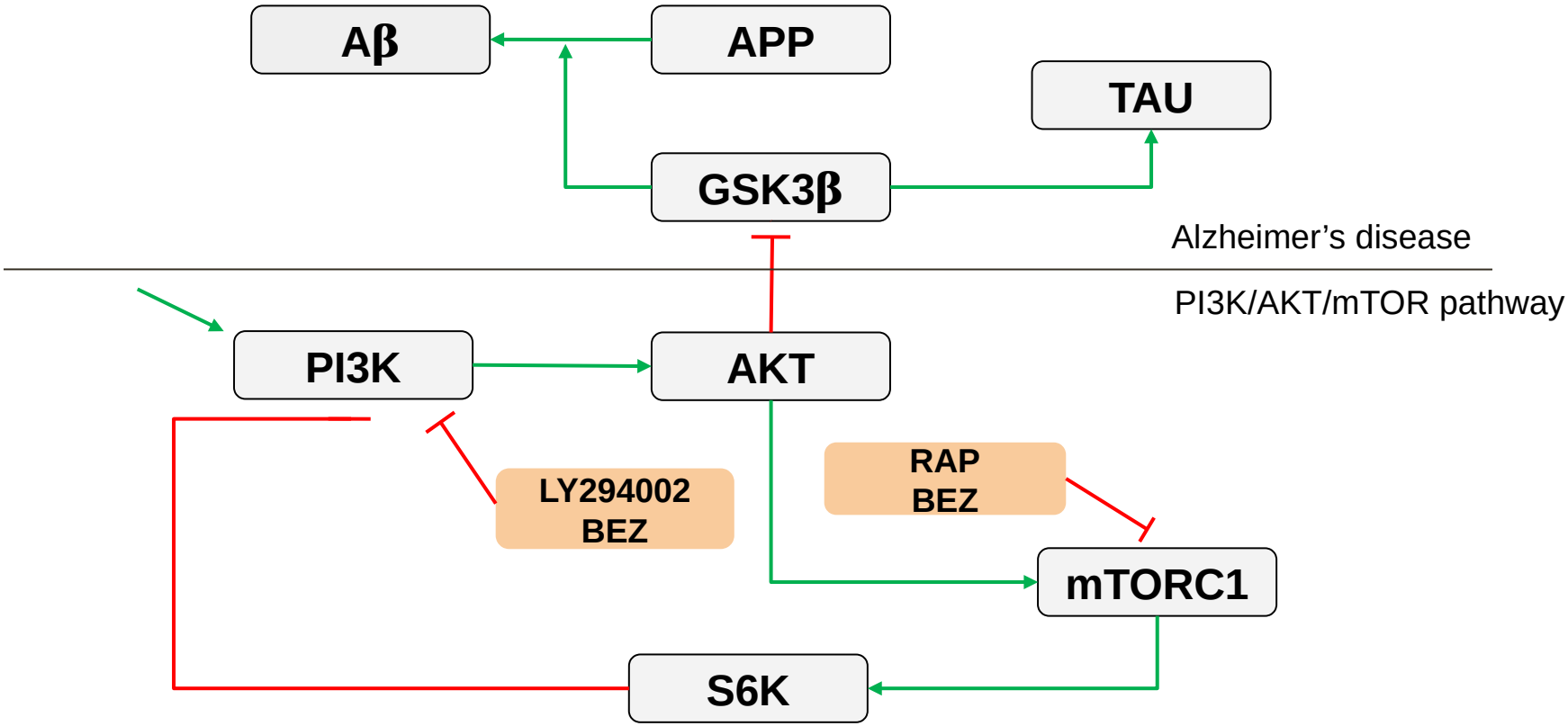
Scope	Model	Variables	Model Type
Alzheimer's Disease	[33]	APP-A β -GSK3 β -Tau-NFT	ODE
	[34]	APP-A β -GSK3 β -Tau-NFT-Neuronal Death	PDE

PI3K/mTOR/Aβ/Tau pathway



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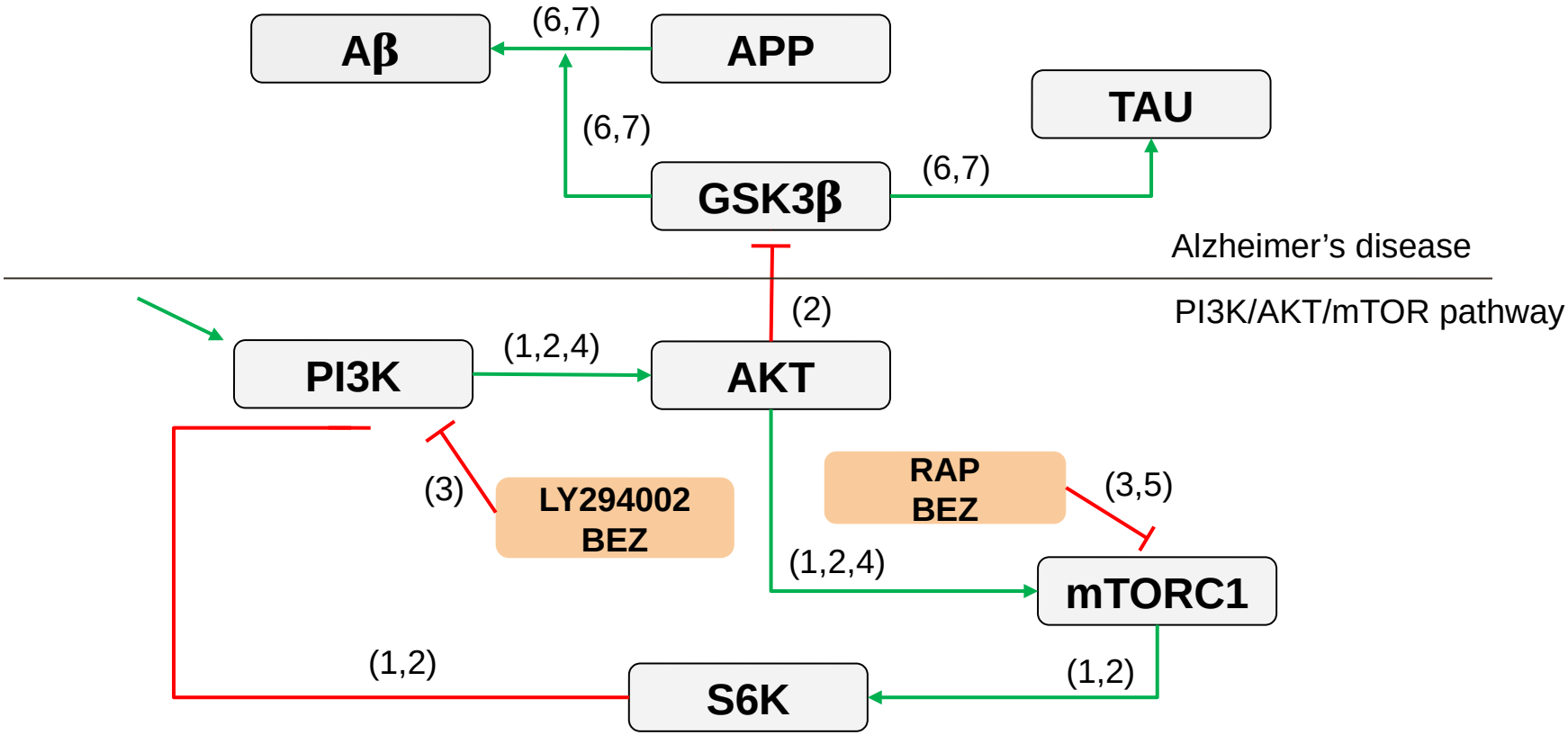
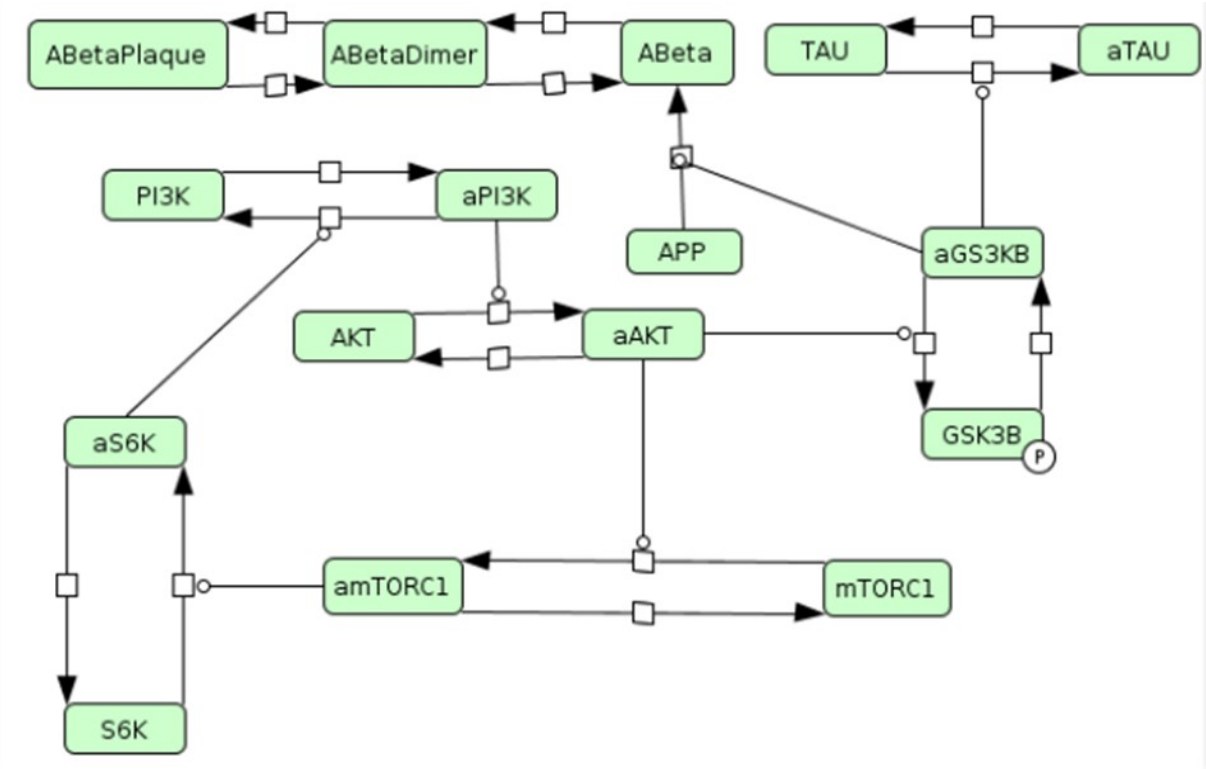
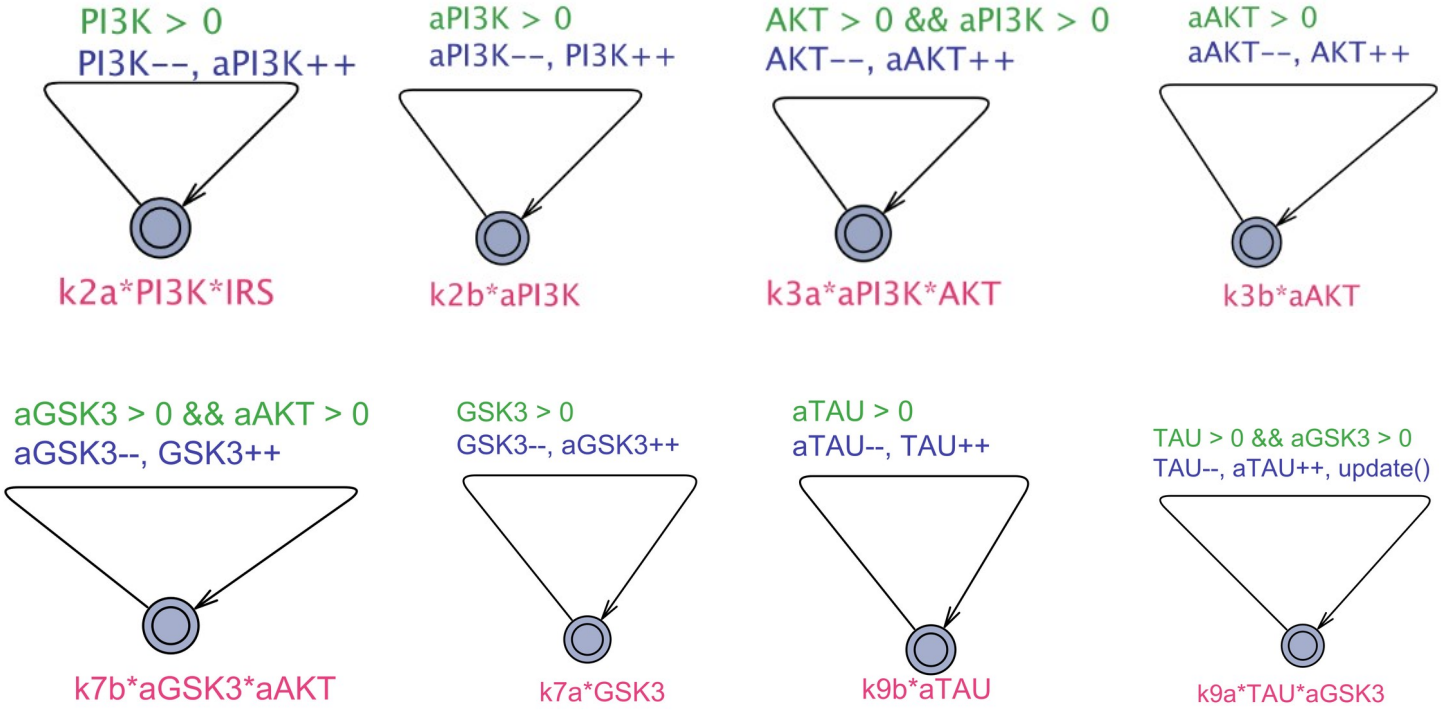
PI3K/mTOR/Aβ/Tau pathway



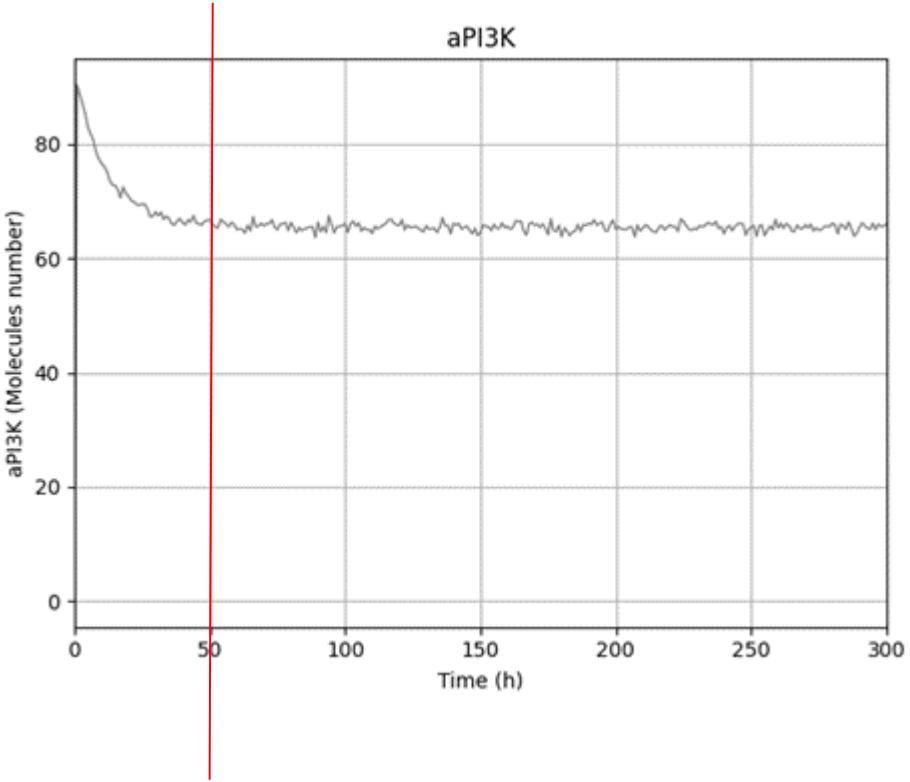
The stochastic formal model

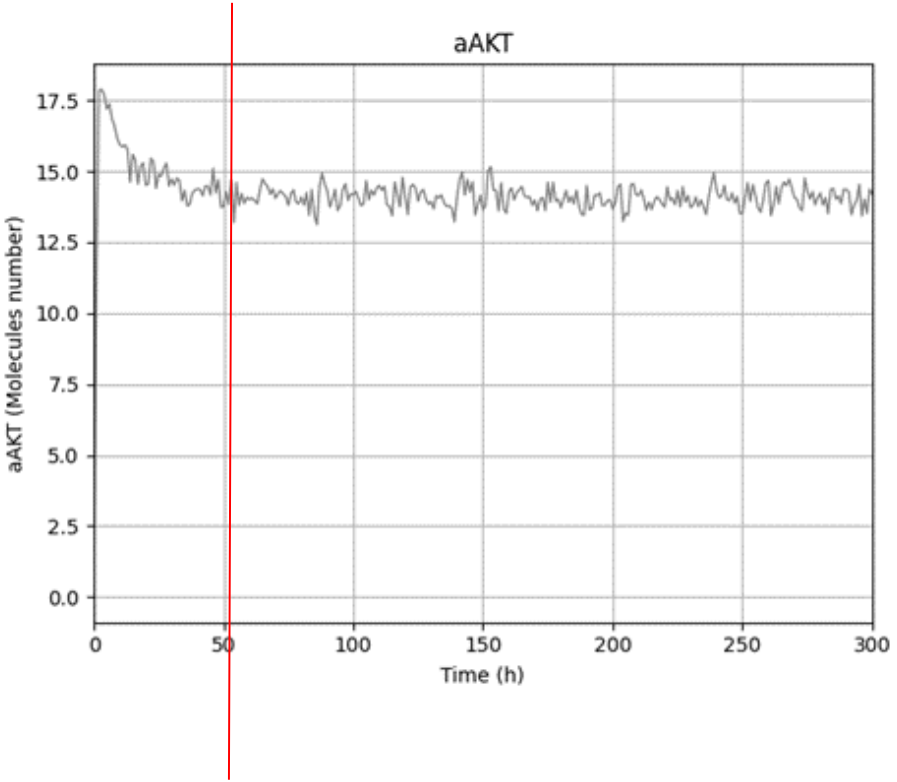


Uppaal – PTAs

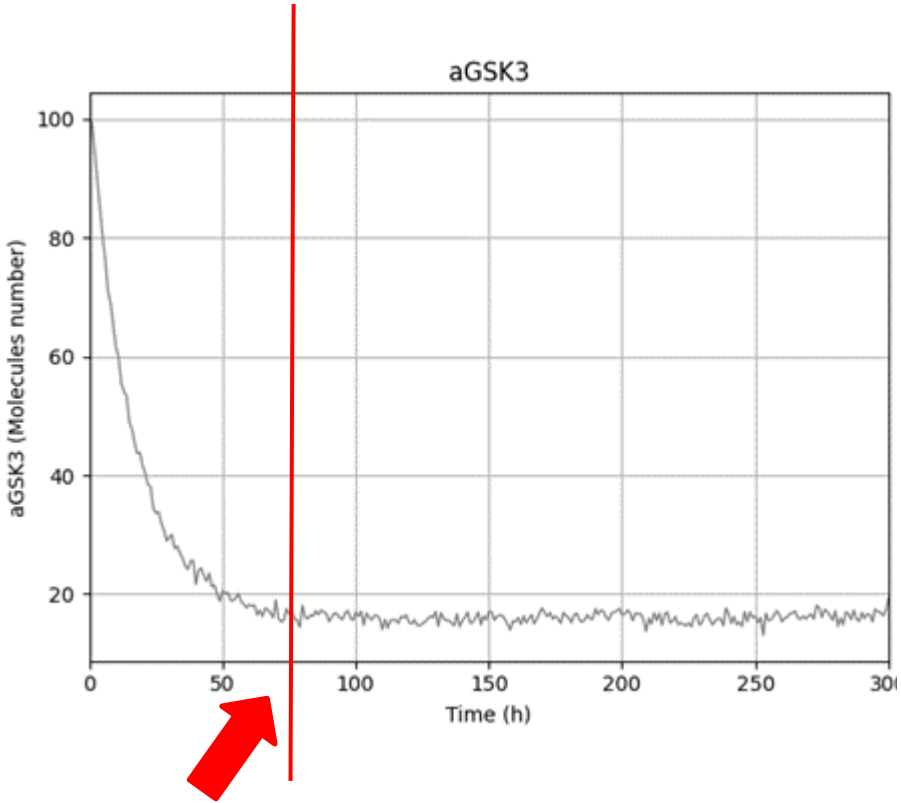

PI3K > 0
PI3K--, aPI3K++
k2a*PI3K*IRS
aPI3K > 0
aPI3K--, PI3K++
k2b*aPI3K
AKT > 0 && aPI3K > 0
AKT--, aAKT++
k3a*aPI3K*AKT
aAKT > 0
aAKT--, AKT++
k3b*aAKT
aGSK3 > 0 && aAKT > 0
aGSK3--, GSK3++
k7b*aGSK3*aAKT
GSK3 > 0
GSK3--, aGSK3++
k7a*GSK3
aTAU > 0
aTAU--, TAU++
k9b*aTAU
TAU > 0 && aGSK3 > 0
TAU--, aTAU++, update()
k9a*TAU*aGSK3

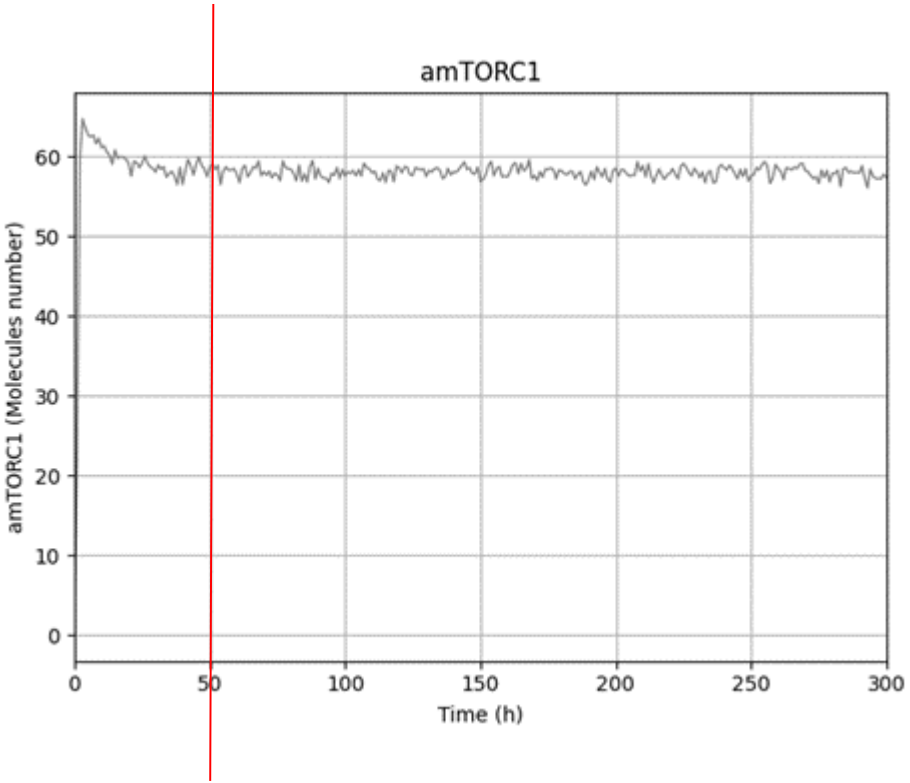
Model simulation



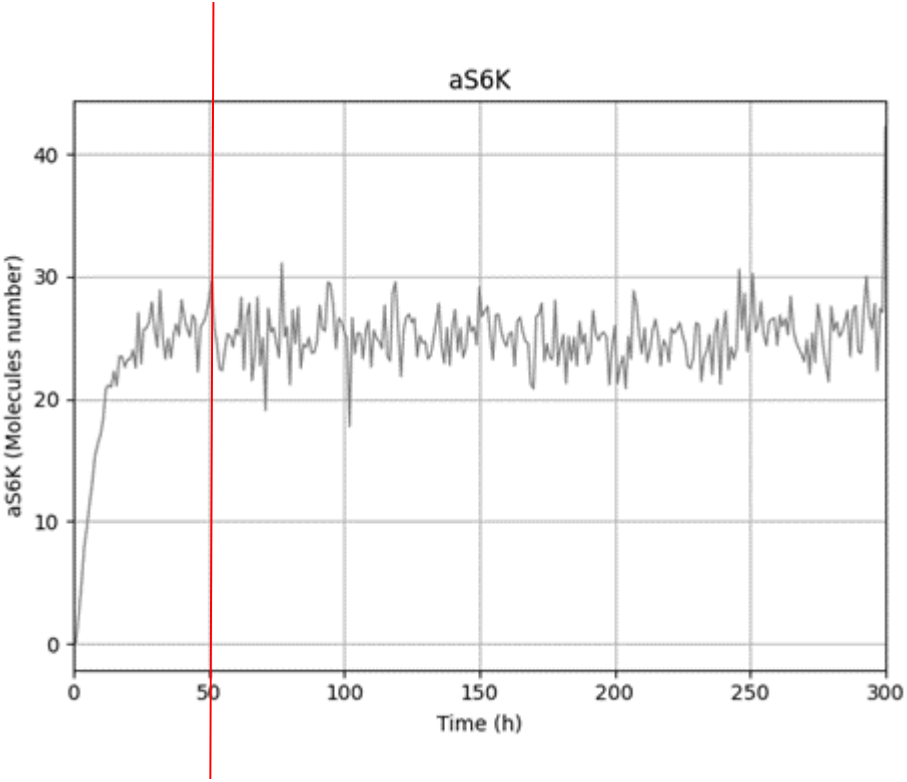


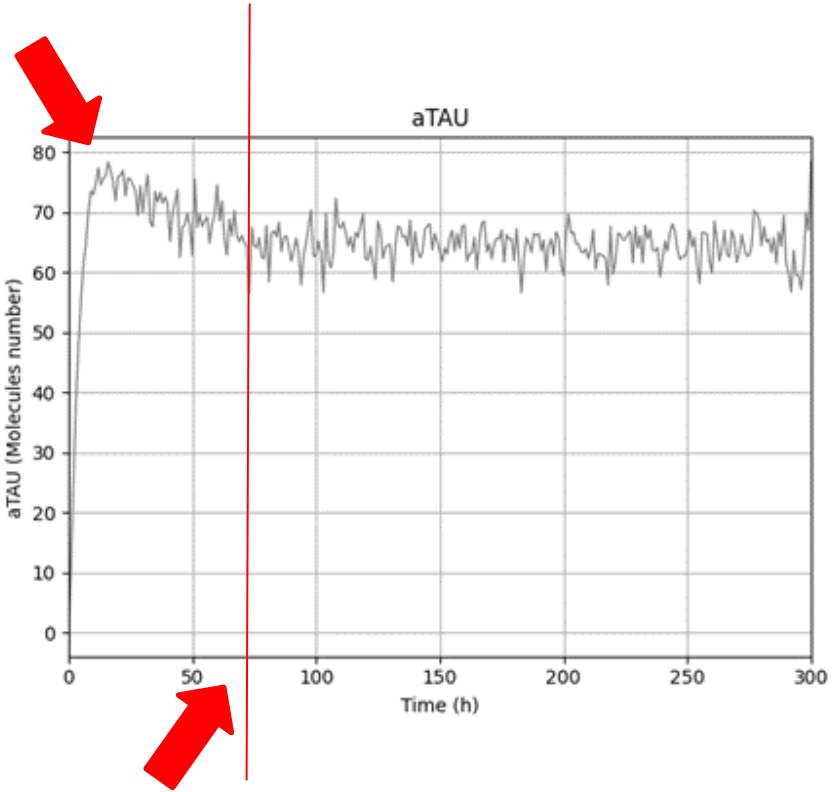
Model simulation





Model simulation





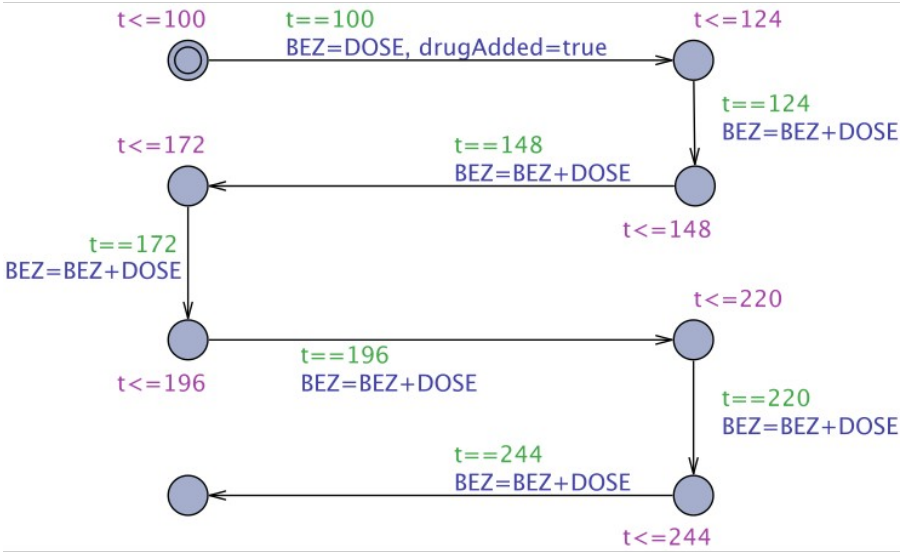
Scenarios

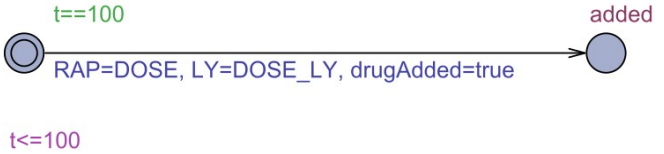
- Control;
- 50 e 500 units of the drug;
- 50 units daily during 1 week, and;
- 50 of LY294002 + 50 units of rapamycin.

Ideals results

- ↓ Aβ formation
- ↓ Tau phosphorylation

Uppaal – PTAs


t<=100
t==100
BEZ=DOSE, drugAdded=true
t<=124
t==124
BEZ=BEZ+DOSE
t<=172
t==148
BEZ=BEZ+DOSE
t<=148
t==172
BEZ=BEZ+DOSE
t<=220
t<=196
t==196
BEZ=BEZ+DOSE
t==220
BEZ=BEZ+DOSE
t==244
BEZ=BEZ+DOSE
t<=244


t==100
added
RAP=DOSE, LY=DOSE_LY, drugAdded=true
t<=100

Properties Verified

1. **What is the likelihood in reducing in 5% the Aβ formation over 300 hours?**

   Pr[<=300] (<> ((drugAdded) && (ABETA < 112*0.95)))

2. **What is the likelihood of tau phosphorylation reduction over 300 hours?**

   Pr[<=300] (<> aTAU > TAUactivation.maxATAU && drugAdded)

3. **What is the likelihood of S6K recovering its normal behavior over 300 hours?**

   Pr[<=300] (<> (drugAdded) && (aS6K > S6Kactivation.maxAS6K))

Results - Reducing 5% on AB production

Table 3
Different scenarios were tested with (RAP), LY294002, their combination, and NVP-BEZ235. The interval represents the likelihood of reducing A β production in 5%. In this case, the highest value is better for Alzheimer's treatment. The probability interval is given considering 95% of confidence level.

Scenario	Number of traces	Time (s)	Probability (%)	
			Inf.	Sup.
Control	383	200	55.56	65.54
50 RAP	375	218	58.36	68.35
500 RAP	384	208	58.09	68.09
50 RAP daily 7 days	381	210	55.02	65.02
50 LY294002	390	387	62.08	72.07
500 LY294002	380	433	62.46	72.44
50 LY294002 daily 7 days	380	901	60.35	70.35
50 LY294002 + 50 RAP	363	334	52.84	62.84
50 NVP-BEZ235	371	252	54.32	64.30
500 NVP-BEZ235	386	216	55.28	65.28
50 NVP-BEZ235 daily 7 days	377	305	52.85	62.85

Results - Tau Phosporylation

Table 4
Different scenarios were tested with rapamycin (RAP), LY294002, their combination, and NVP-BEZ235. The interval represents the likelihood of tau phosphorylation increasing. In this case, the lower value is better for Alzheimer's treatment. The probability interval is given considering 95% of confidence level.

Scenario	Number of traces	Time (s)	Probability (%)	
			Inf.	Sup.
Control	245	195	9.94	19.94
50 RAP	217	205	7.12	17.09
500 RAP	179	153	5.19	15.16
50 RAP daily 7 days	217	182	7.73	17.72
50 LY294002	291	516	17.09	27.08
500 LY294002	338	723	27.55	37.53
50 LY294002 daily 7 days	386	1408	48.98	58.96
50 LY294002 + 50 RAP	324	583	18.74	28.73
50 NVP-BEZ235	326	356	18.92	19.95
500 NVP-BEZ235	124	94	85.89	95.86
50 NVP-BEZ235 daily 7 days	79	78	90.19	100

Discussion

Table 6

Summary of the response of drug administration on each variable. The ideal scenario is a strategy capable of reducing A β production and tau phosphorylation. None of the scenarios tested in our model achieved such a result.

Scenario	A β formation	tau phosphorylation
Ideal	↓	↓
50 RAP	-	↓
500 RAP	-	↓
50 RAP daily 7 days	-	↓
50 LY294002	↓	↑
500 LY294002	↓	↑
50 LY294002 daily 7 days	↓	↑
50 LY294002 + 50 RAP	↓	↑
50 NVP-BEZ235	-	↑
500 NVP-BEZ235	-	↑
50 NVP-BEZ235 daily 7 days	-	↑

Discussion

- Formal stochastic model of the PI3K/AKT/mTOR pathway and Aβ formation and Tau phosphorylation.
  - The automata can be easily adjusted for new checks.
- It allowed identifying behaviors that were not observed with the simulations and graphic visualization.
- Good response time when checking properties.
  - Personal computer (i3, 8GB de RAM).

Conclusion

- Alzheimer's disease has major challenges to overcome.
- Using SMC, we show the impacts of rapamycin, LY294002, and NVP-BEZ235 on Alzheimer's Disease.
  - High doses do not result in improvement.
  - Inhibition of PI3K may contribute to an increase in Tau phosphorylation.
- The SMC technique proved to be efficient in systems analysis.
- We expect that our approach will assist in the experimental design of pharmacological strategies.

Future work

- Animal experiments to evaluate the impact of BEZ235 on Tau phosphorylation.
- Identify other potential drugs for evaluation.
- Model expansion adding the autophagy process of amyloid plaques.

Statistical Model Checking no reposicionamento de drogas na Doença de Alzheimer

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