Inserm

Institut national
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Mount Sinai

SYSTEMS CANCER CHRONOTHERAPEUTICS : IN VITRO MOLECULAR CHRONOPHARMACOLOGY OF THE ANTICANCER DRUG IRINOTECAN

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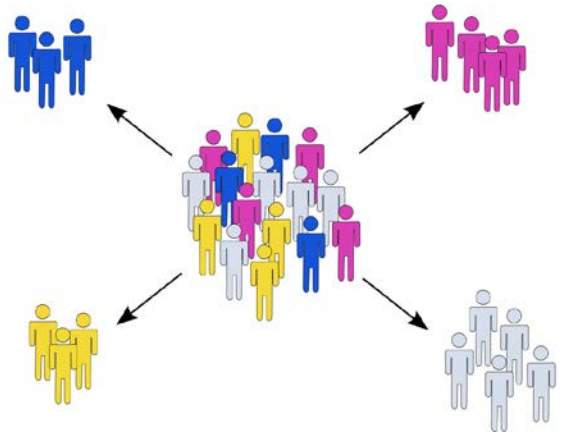
OUTLINE

1. In vitro molecular chronopharmacology of irinotecan
2. Personalizing irinotecan circadian delivery: a tutorial in Scilab.

Molecular-based personalization of cancer chronotherapeutics

- Recent clinical evidence of the **need for personalized** chronomodulated administration scheme.



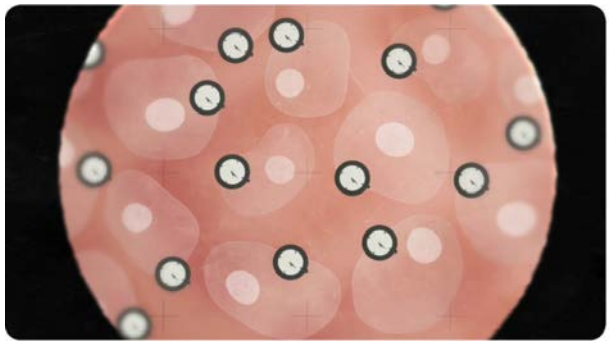
- Require molecular **understanding of drug chronoPK-PD** (pharmacokinetics-pharmacodynamics) towards identification of potential **clinical biomarkers.**

- **In vitro combined biological and mathematical** study of irinotecan (CPT11) molecular chronoPK-PD

In vitro circadian studies

- Rationale of in vitro studies: every nucleated cell endowed with a molecular circadian clock.

- In the absence of synchronizer, clocks do not display the same period or phase.
- Seric shocks (ie. exposing cells to a large amount of nutrients during 2 hours) synchronize the circadian clock of the cells which oscillate in synchrony:



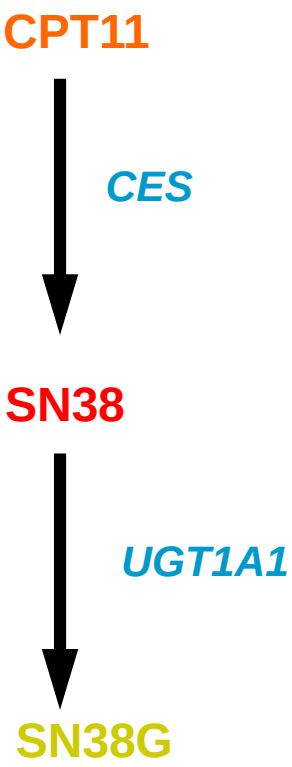
- Choice of **Caco-2 human colorectal cancer cells for irinotecan study:** well–established in vitro model for human intestine + express clock genes

Irinotecan (CPT11)

- Clinically-approved for the treatment of colorectal cancer since 1994.

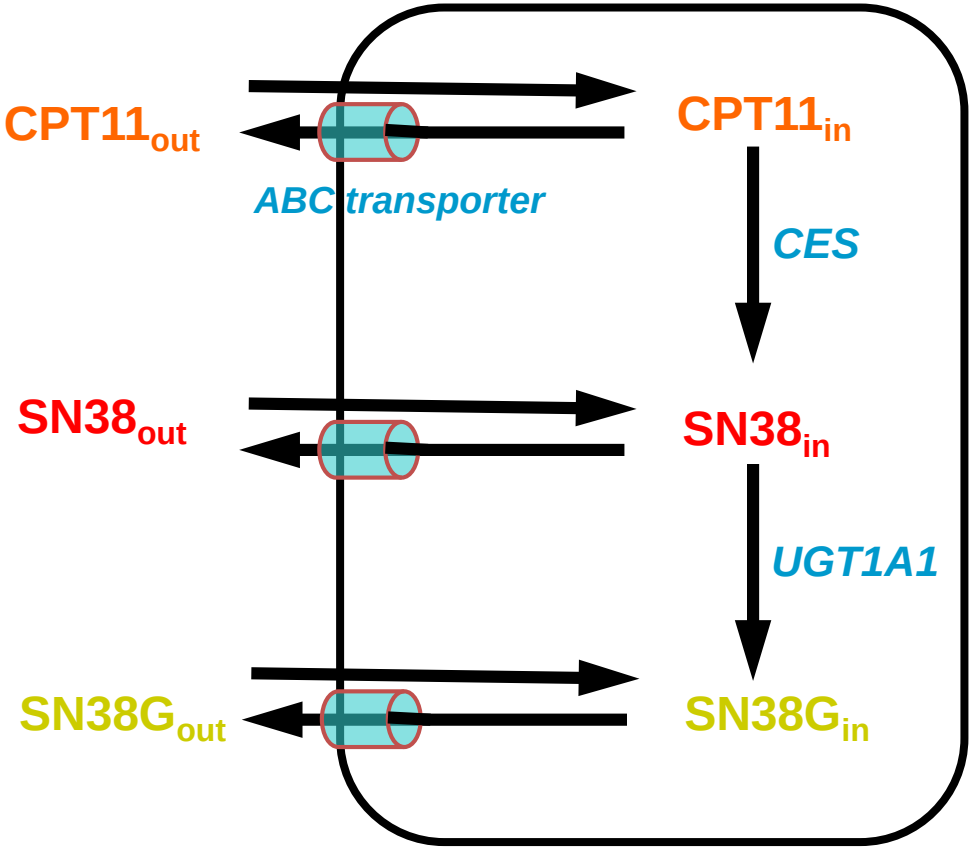
- Part of the **camptothecin** family. Active principle comes from the chinese tree *Camptotheca acuminata decne.*

CPT11 Pharmacokinetics (PK)



- **CPT11** is bioactivated into **SN38** by carboxylesterases (**CES**). **SN38** is 100 to 1000 fold more cytotoxic than **CPT11.**
- **SN38** is transformed into **SN38G** mainly under the enzymatic activity of **UGT1A1**. **SN38G** is completely inactive.

CPT11 Pharmacokinetics (PK)



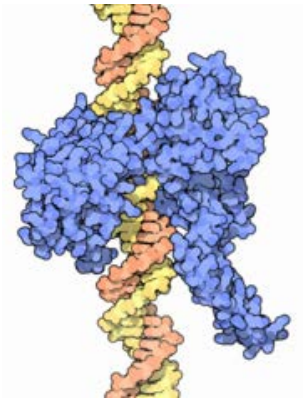
- **CPT11**, **SN38** and **SN38G** are pumped outside of the cells **by ABC transporters** (ABC= ATP-Binding Cassette)

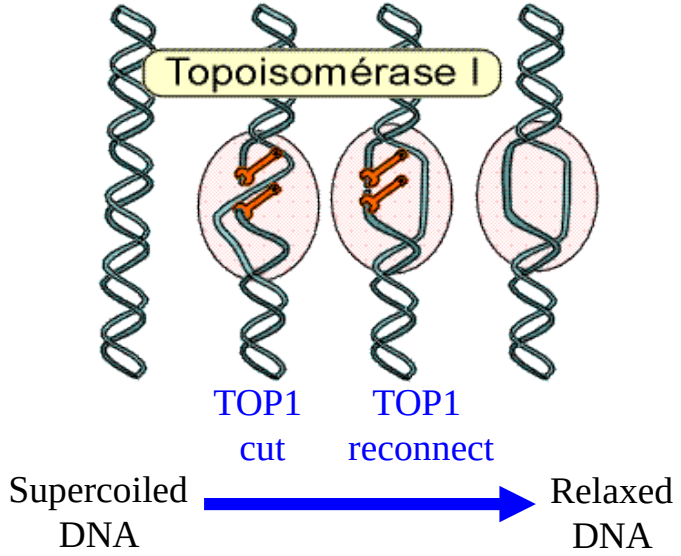
CPT11 Pharmacodynamics (PD)

- CPT11 is an inhibitor of topoisomerase 1 (TOP1).
- What is TOP1? An enzyme present in all nucleated cells whose function is to relax supercoiled DNA.

TOP1

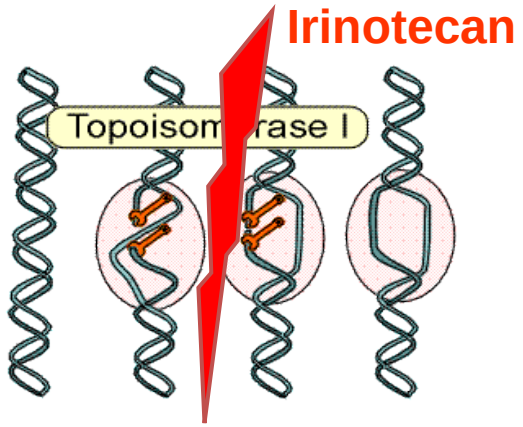
DNA



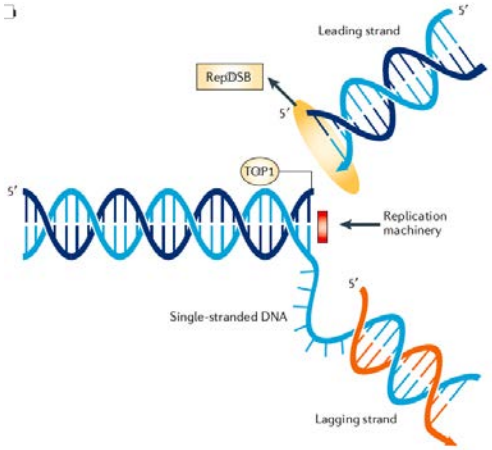


CPT11 Pharmacodynamics (PD)

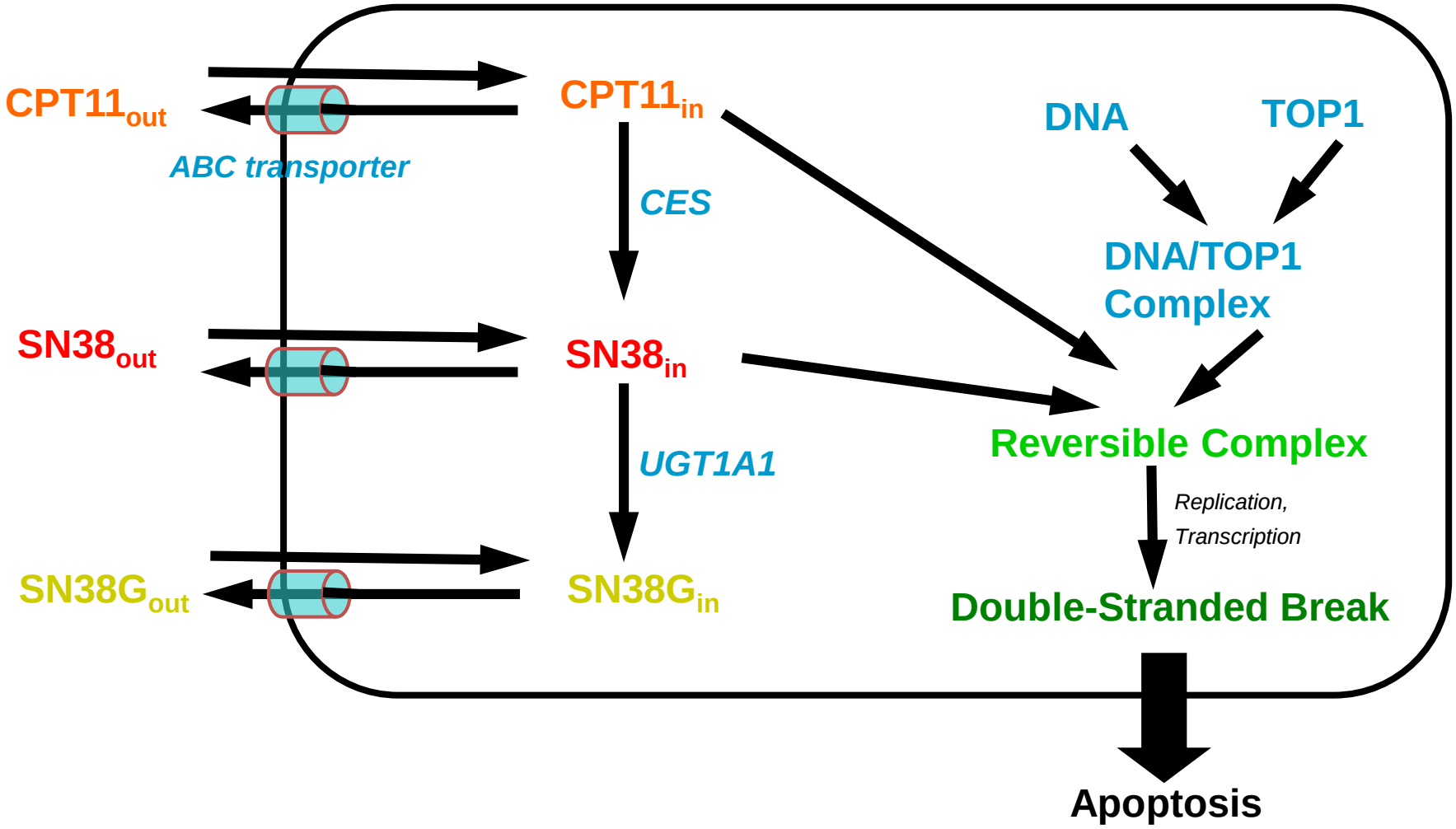
- Irinotecan **prevents TOP1 from reconnecting the broken DNA strand**, creating reversible TOP1/DNA/Irinotecan complexes.



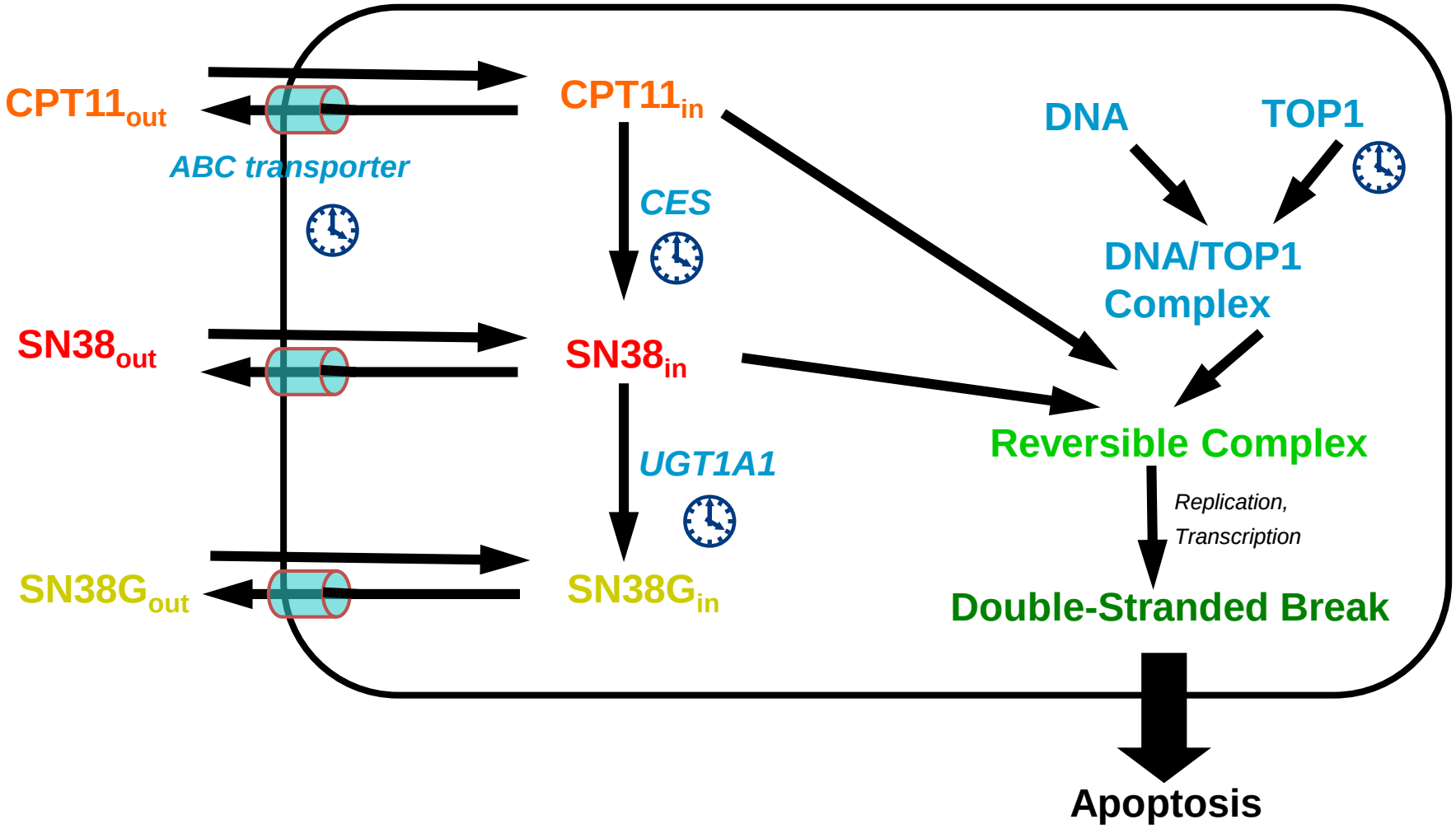
- **Collisions** between those complexes and replication forks or transcription mechanisms create **DNA double-stranded breaks**, which can be lethal for the cell.



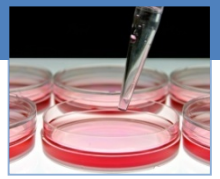
CPT11 molecular PK-PD



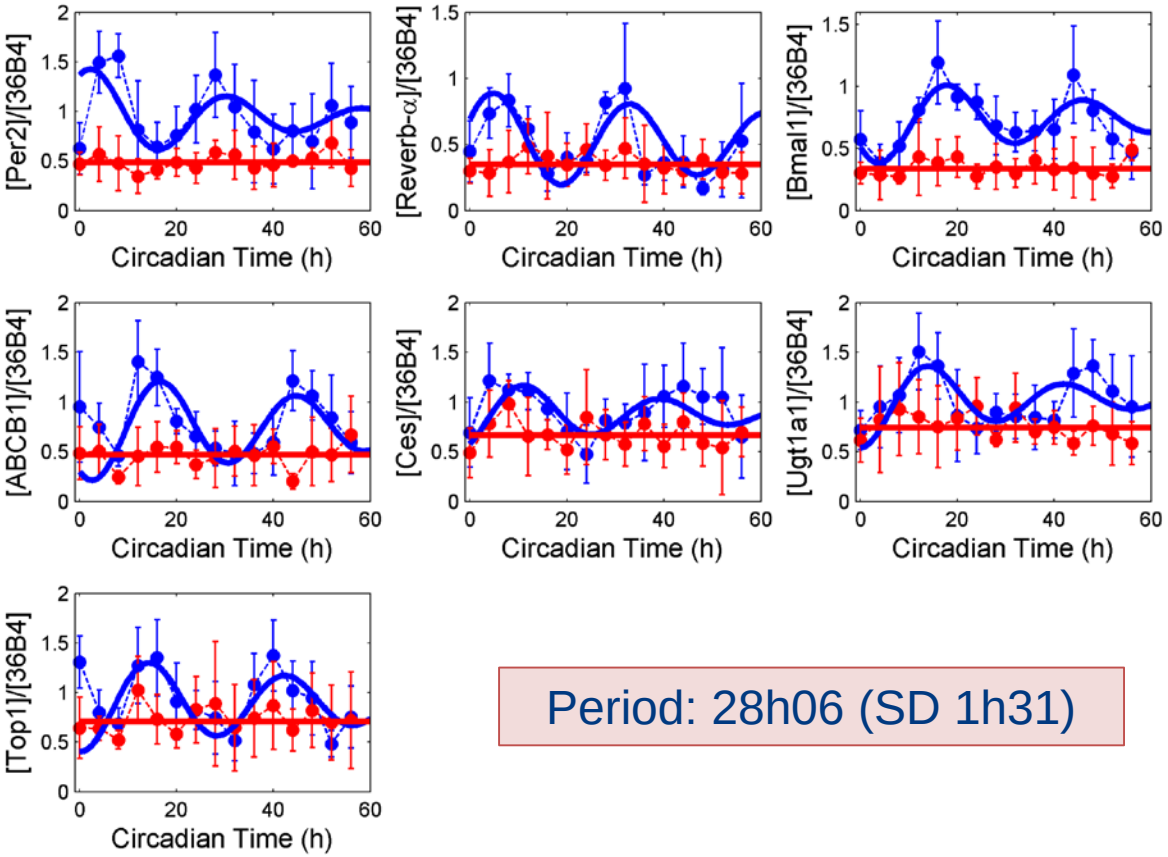
CPT11 molecular PK-PD



IRINOTECAN MOLECULAR CHRONOPHARMACOLOGY IN CACO-2 CELLS

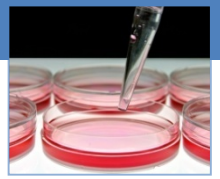


mRNA circadian rhythms

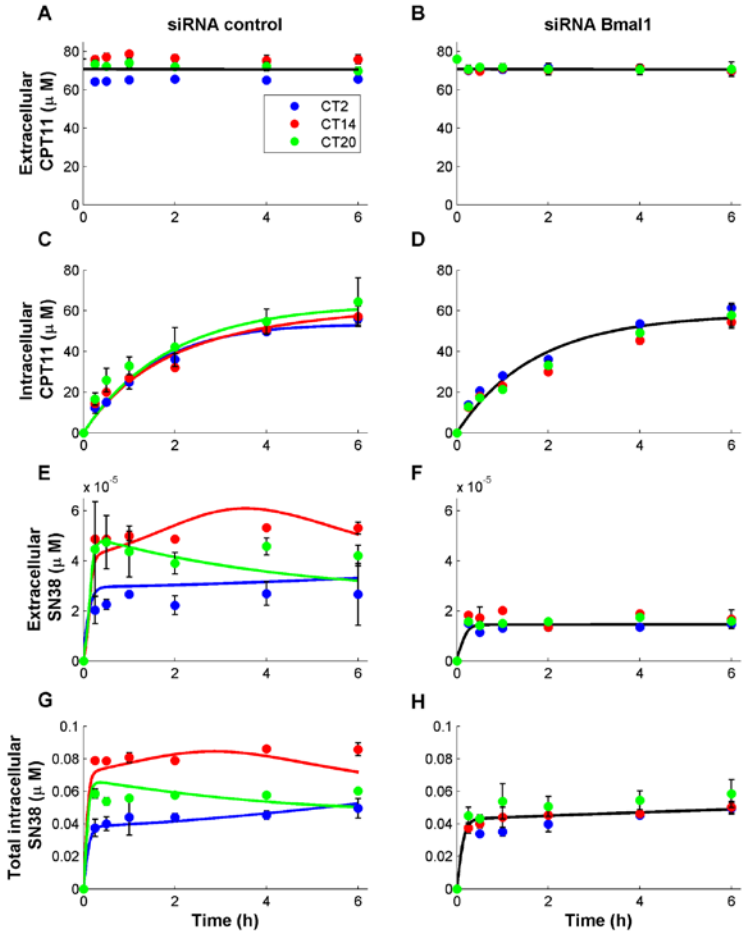


Period: 28h06 (SD 1h31)

- Circadian variations of mRNA expression of 3 clock genes and 4 drug metabolism genes in synchronized Caco-2 cells.
- …disrupted in clock-deficent cells (siRNA Bmal1).

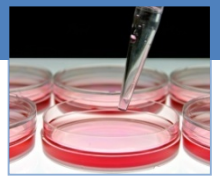


CPT11 PK in Caco-2 cells

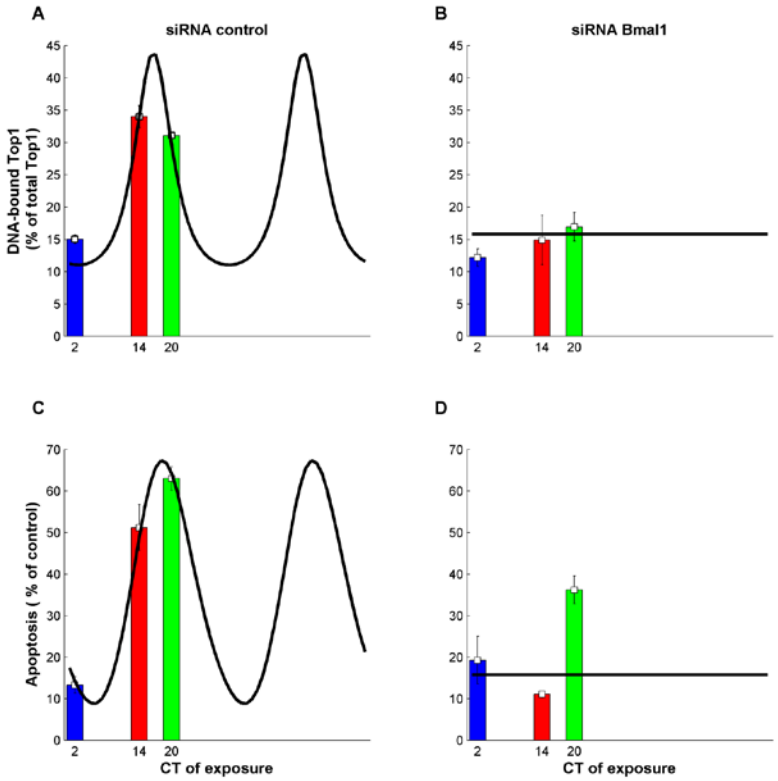


Cells exposed to CPT11 (76 μM) during 6h.

- Large and statistically significant circadian variations of SN38in and out in the presence of functional clock.
- …which are disrupted in clock-deficient cells



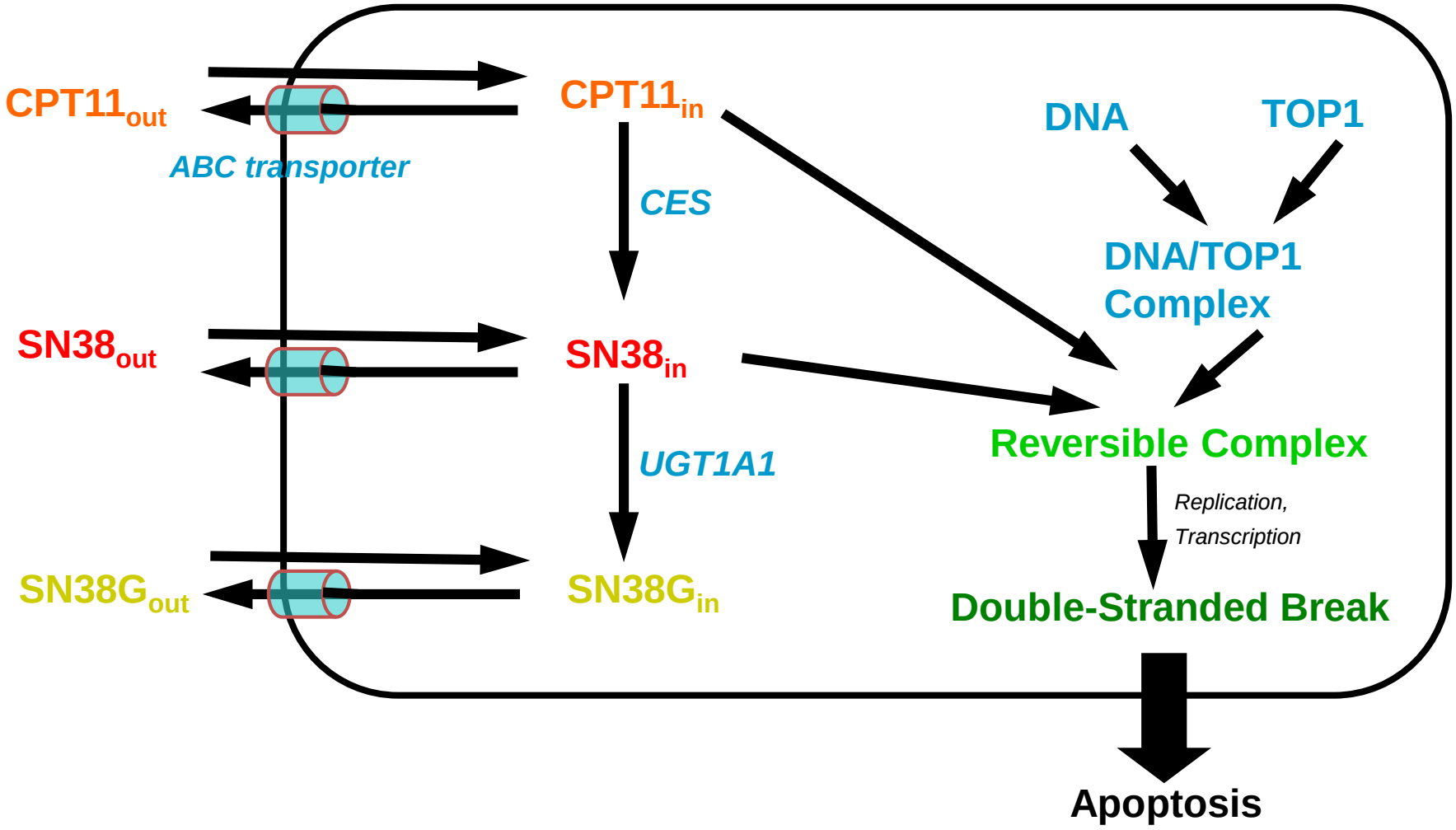
Circadian rhythms of CPT11 PD



- Circadian variations of % of DNA-bound TOP1 (PD marker) and apoptosis in the presence of irinotecan
- …disrupted in clock-deficent cells.

MODEL OF IRINOTECAN MOLECULAR CHRONO PK-PD

CPT11 molecular PK-PD



Ordinary differential equations

$$\frac{d[CPT_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{upCPT}[CPT_{out}] + \frac{V_{effCPT}[ABC\_CPT][CPT_{in}]}{K_{effCPT} + [CPT_{in}]}$$

$$\frac{d[CPT_{in}]}{dt} = k_{upCPT}[CPT_{out}] - \frac{V_{effCPT}[ABC\_CPT][CPT_{in}]}{K_{effCPT} + [CPT_{in}]} - \frac{V_{ces}[CES][CPT11_{in}]}{K_{ces} + [CPT11_{in}]}$$

$$\frac{d[SN_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{upSN}[SN_{out}] + \frac{V_{effSN}[ABC\_SN][SN_{in}]}{K_{effSN} + [SN_{in}]}$$

$$\frac{d[SN_{in}]}{dt} = k_{upSN}[SN_{out}] - \frac{V_{effSN}[ABC\_SN][SN_{in}]}{K_{effSN} + [SN_{in}]} + \frac{V_{ces}[CES][CPT_{in}]}{K_{ces} + [CPT_{in}]} - \frac{V_{ugt}[UGT][SN_{in}]}{K_{ugt} + [SN_{in}]} - k_{f2}[DNATOP1][SN38_{in}] + k_{r2}[Compl]$$

$$\frac{d[TOP1]}{dt} = k_{ftop} - k_{dtop}[TOP1] - k_{f1}[TOP1][DNA_{free}] + k_{r1}[DNA/TOP1] + k_{r2}[Compl]$$

$$\frac{d[DNA/TOP1]}{dt} = k_{f1}[TOP1][DNA_{free}] - k_{f2}[DNA/TOP1][SN_{in}] - k_{r1}[DNA/TOP1]$$

$$\frac{d[Compl]}{dt} = k_{f2}[DNA/TOP1][SN_{in}] - k_{r2}[Compl] - k_{Irr}[Compl]$$

$$\frac{d[Icompl]}{dt} = k_{Irr}[Compl]$$

Mathematical model for protein circadian rhythm

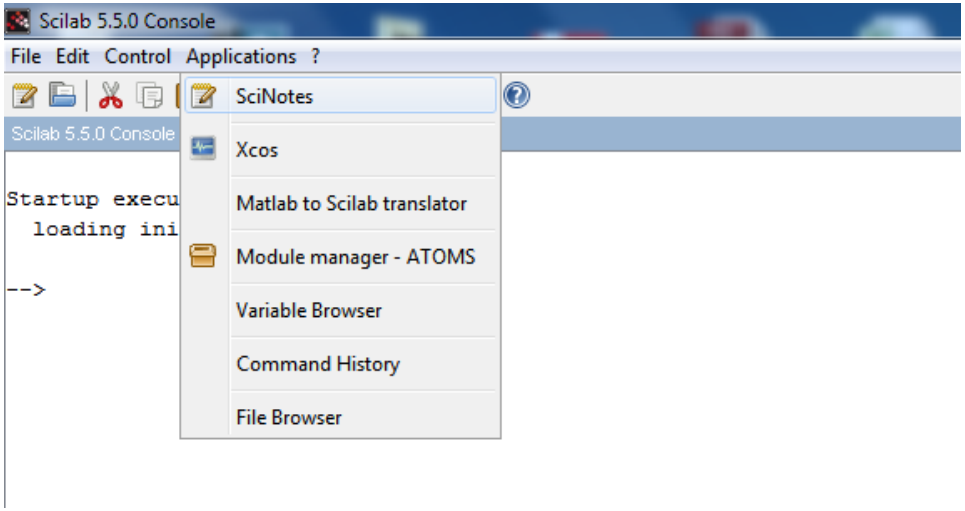
- Equation for protein concentrations:

$$[Protein] = M + Acos(\frac{2\pi}{T}(t-\phi)) .$$

- Parameter estimation utilizing Caco-2 cell datasets, through least square method and Monte carlo simulations (Matlab)

Scilab Tutorial

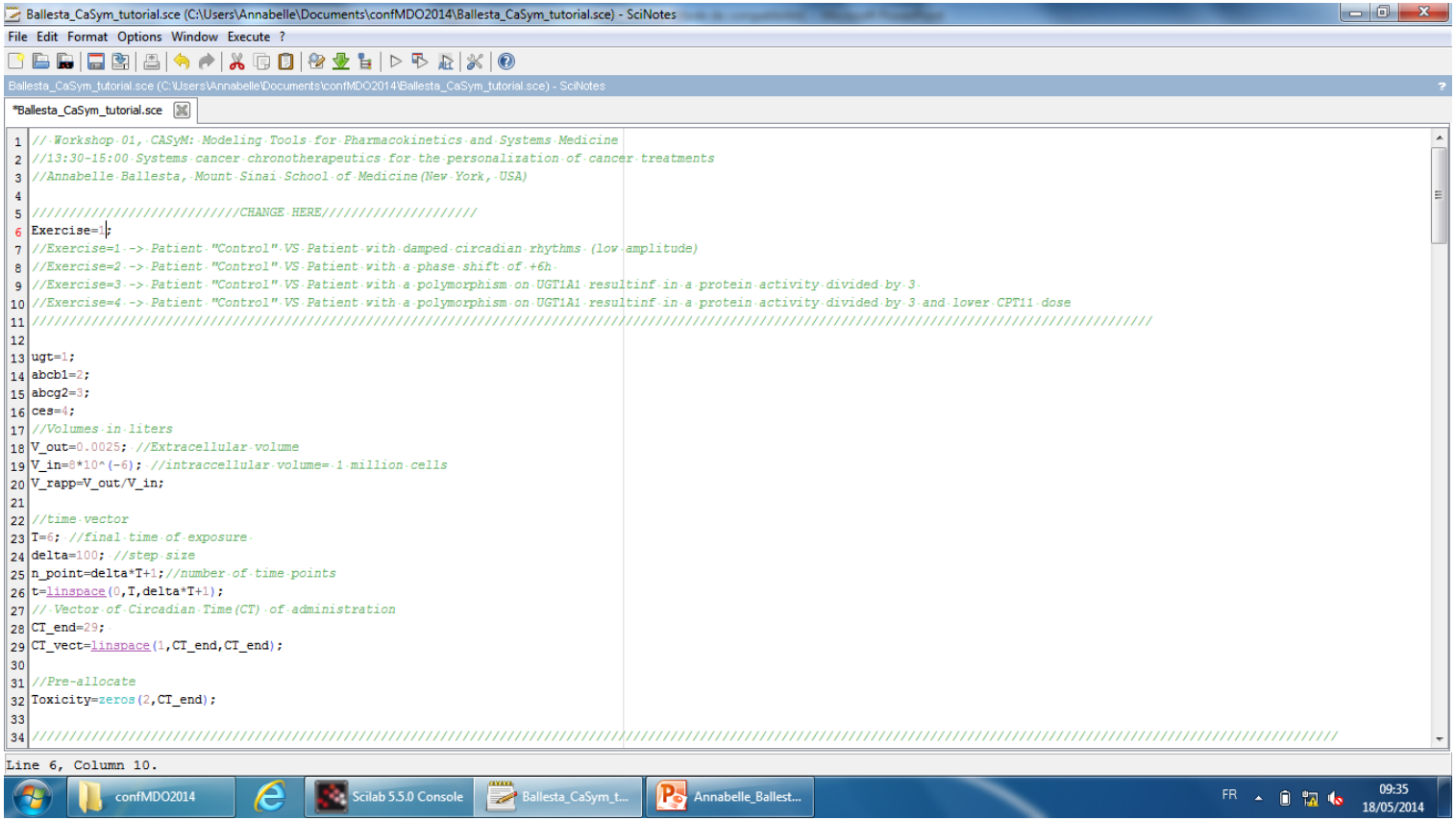
- Download file from: **http://dl.free.fr/mVDfpR5va**
- Open Scilab (Start -> Scilab)
- Open Scinotes : Applications->Scinotes



- Open file in Scinotes

Scilab Tutorial

Desktop should look like this:



Scilab Tutorial

Aim: Demonstrate the need of personalization through modeling.

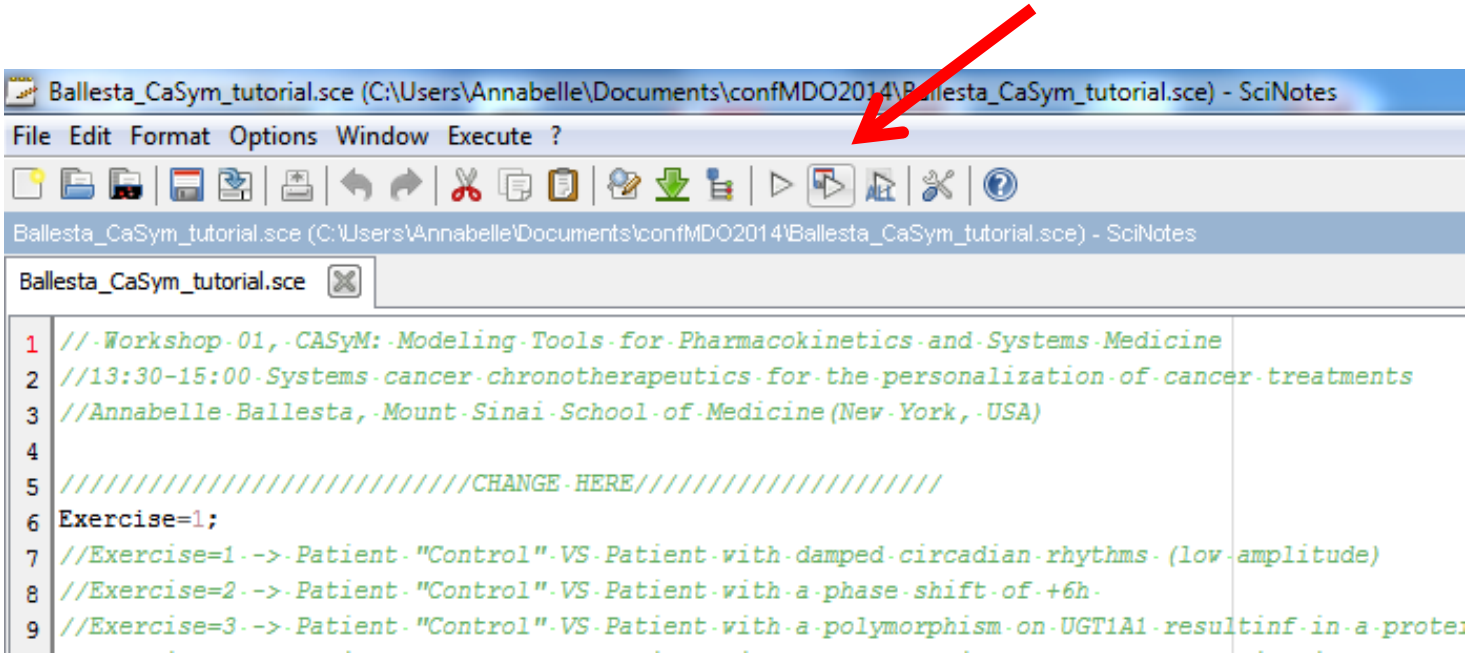
Definitions:

- Irinotecan chronotoxicity = sum of reversible+irreversible complexes.
- Exposure to CPT11 (100 µM) during 6h.
- Patient control= parameters estimated from Caco-2 cells experiments

Scilab Tutorial

Exercise 1: Compare CPT11 chronotoxicity for Patient Control VS Patient with damped circadian rhythms = all amplitudes divided by 2 compared to control

- **Run the file**: press F5 or Click the button:



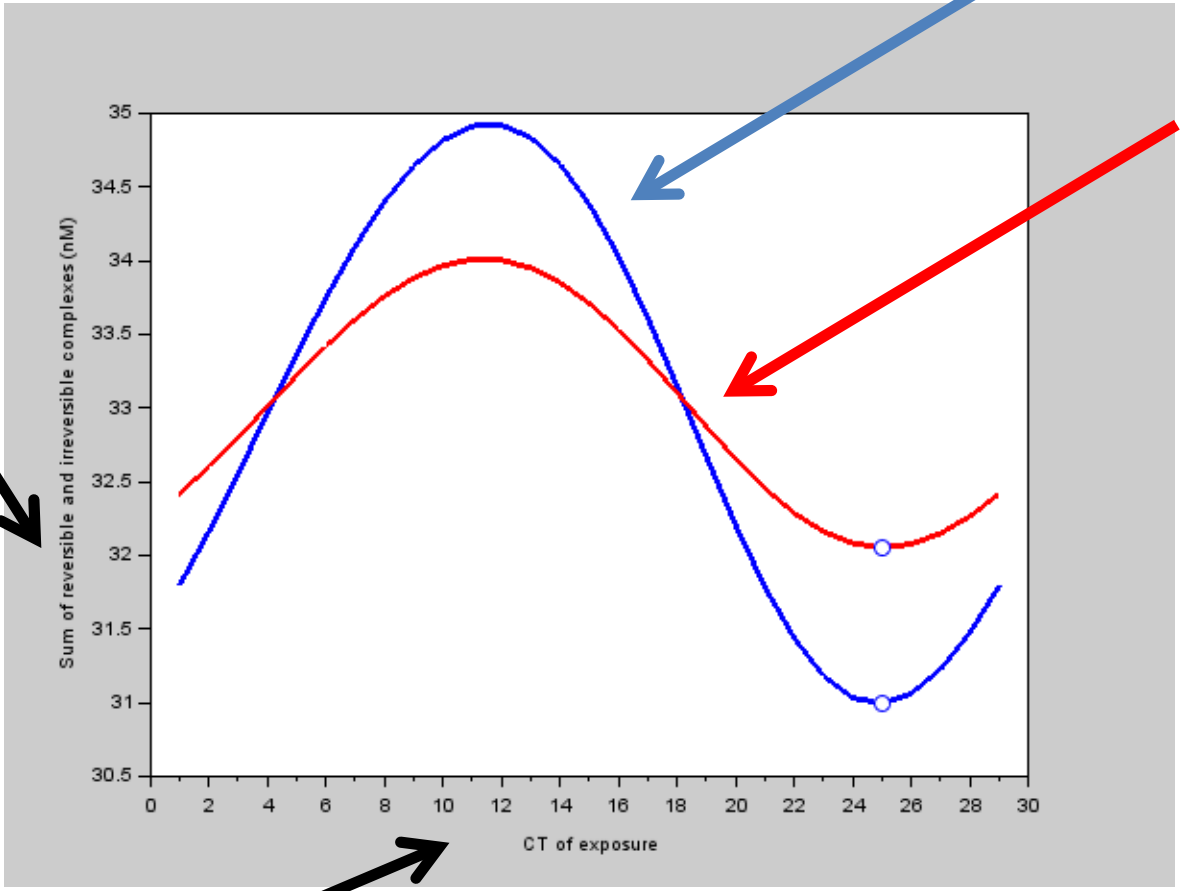
Scilab Tutorial: Exercise 1

Graphical window open:

Patient control

Patient with lower amplitude

Toxicity



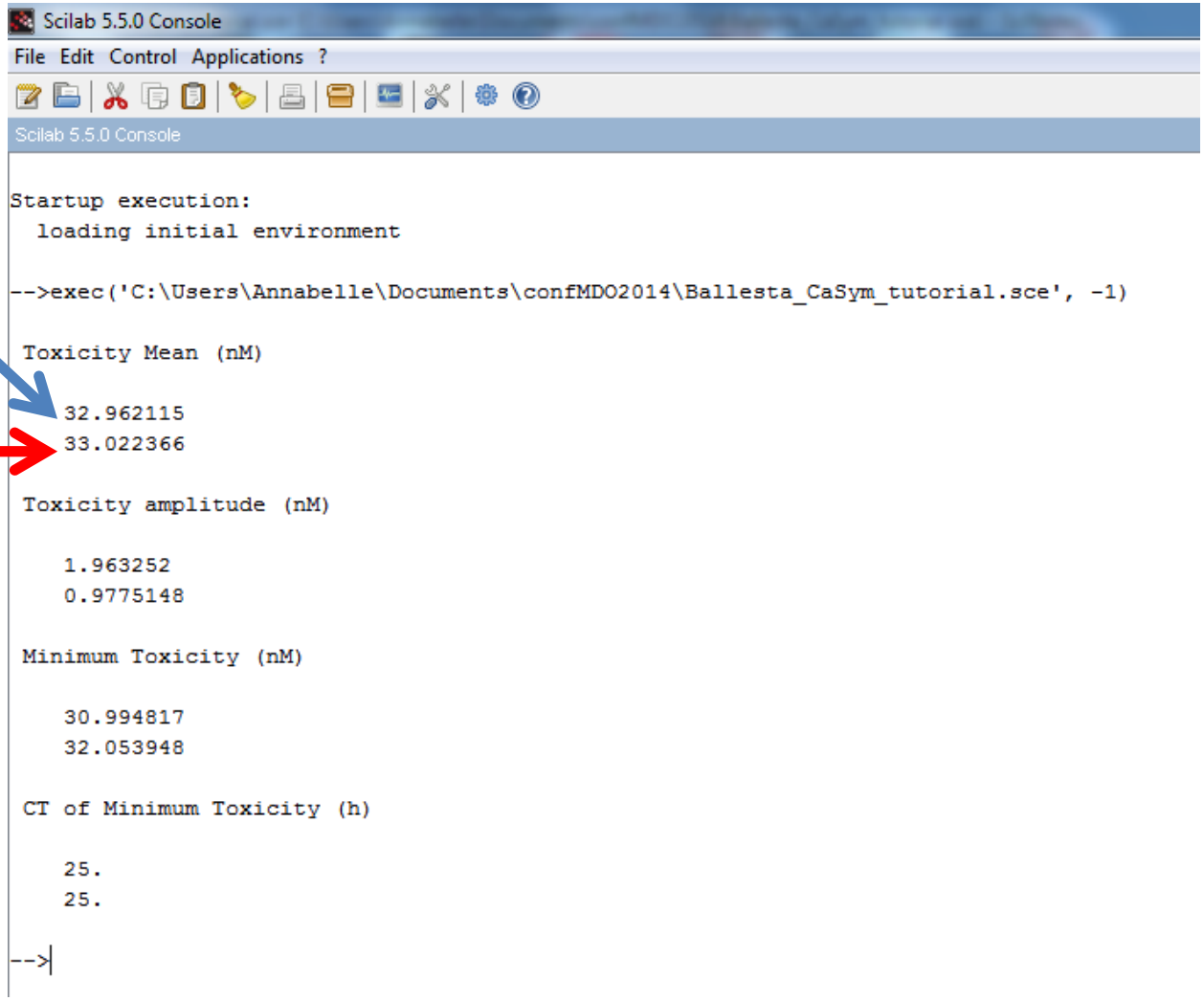
Circadian Time of beginning of exposure

Scilab Tutorial: exercise 1

In the Scilab console:

Patient control

Patient with lower amplitude

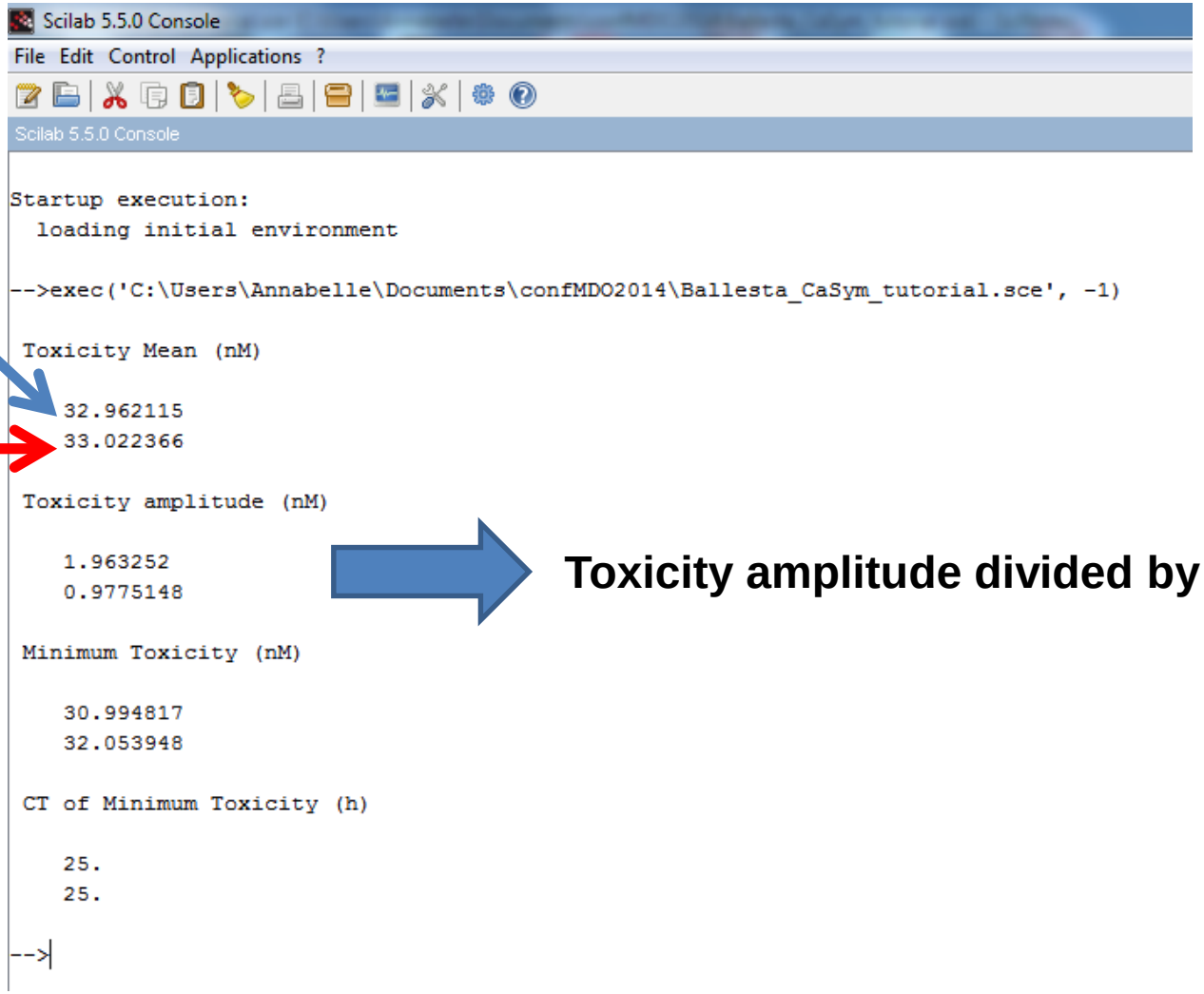


Scilab Tutorial: exercise 1

In the Scilab console:

Patient control

Patient with lower amplitude



Toxicity amplitude divided by 2

Scilab Tutorial: Exercise 2

Exercise 2: Patient Control VS Patient with phase shift =all phases shifted of +6h compared to control.

- In Scinotes: change variable Exercise to 2

Scilab Tutorial: Exercise 2

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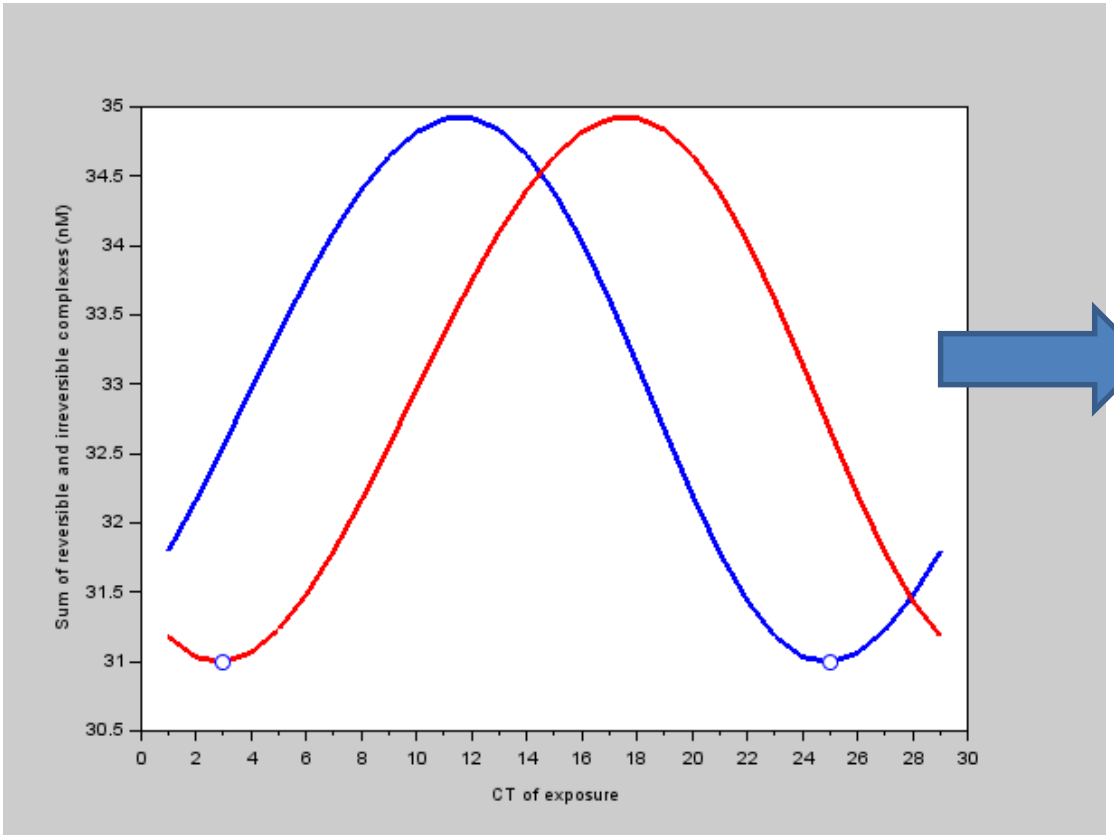
- In Scinotes: change variable Exercise to 2



Toxicity shifted by +6h (modulo the 28.1h period)

Scilab Tutorial

Exercise 3: Patient Control VS Patient with UGT1A1 polymorphism leading to a division by 3 of the protein activity.

Scilab Tutorial

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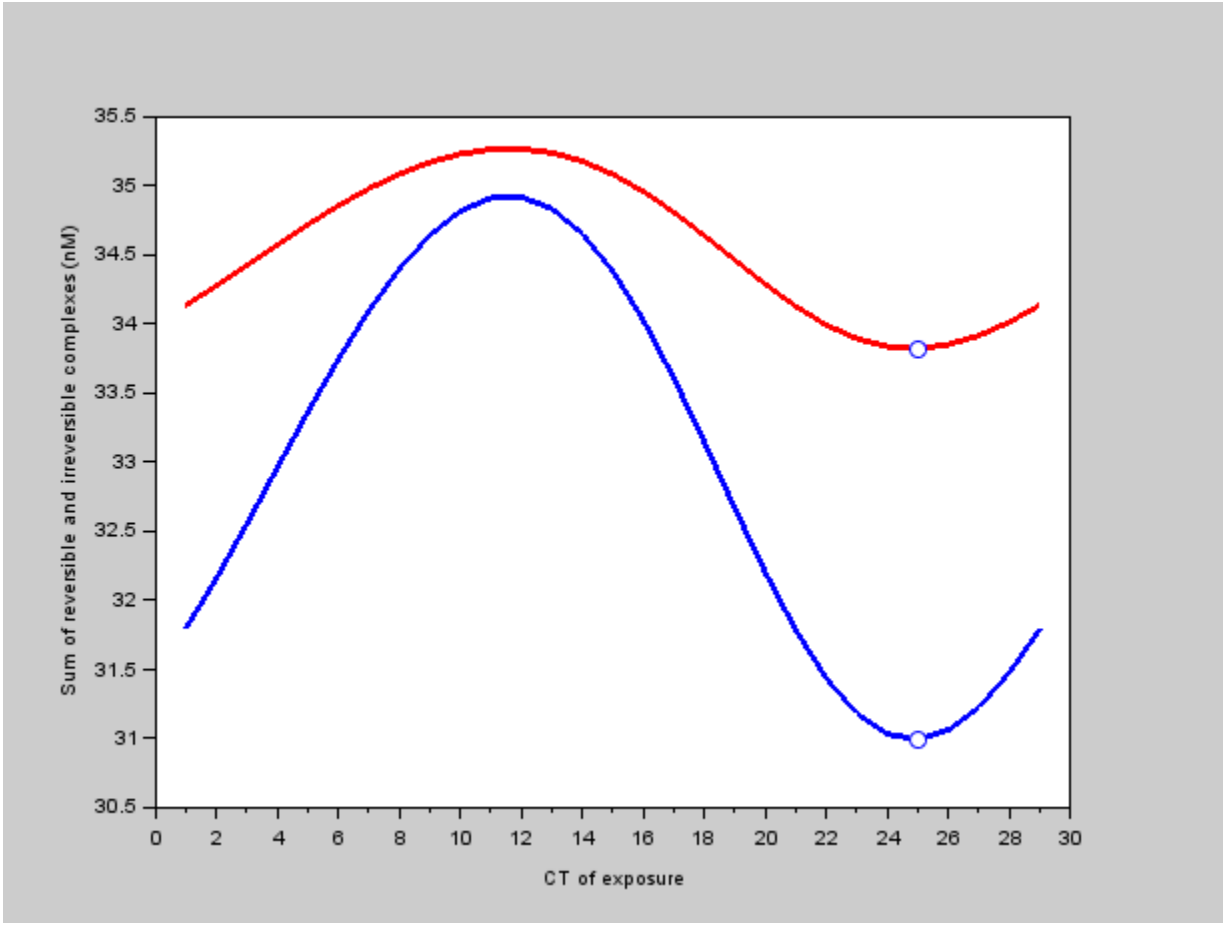
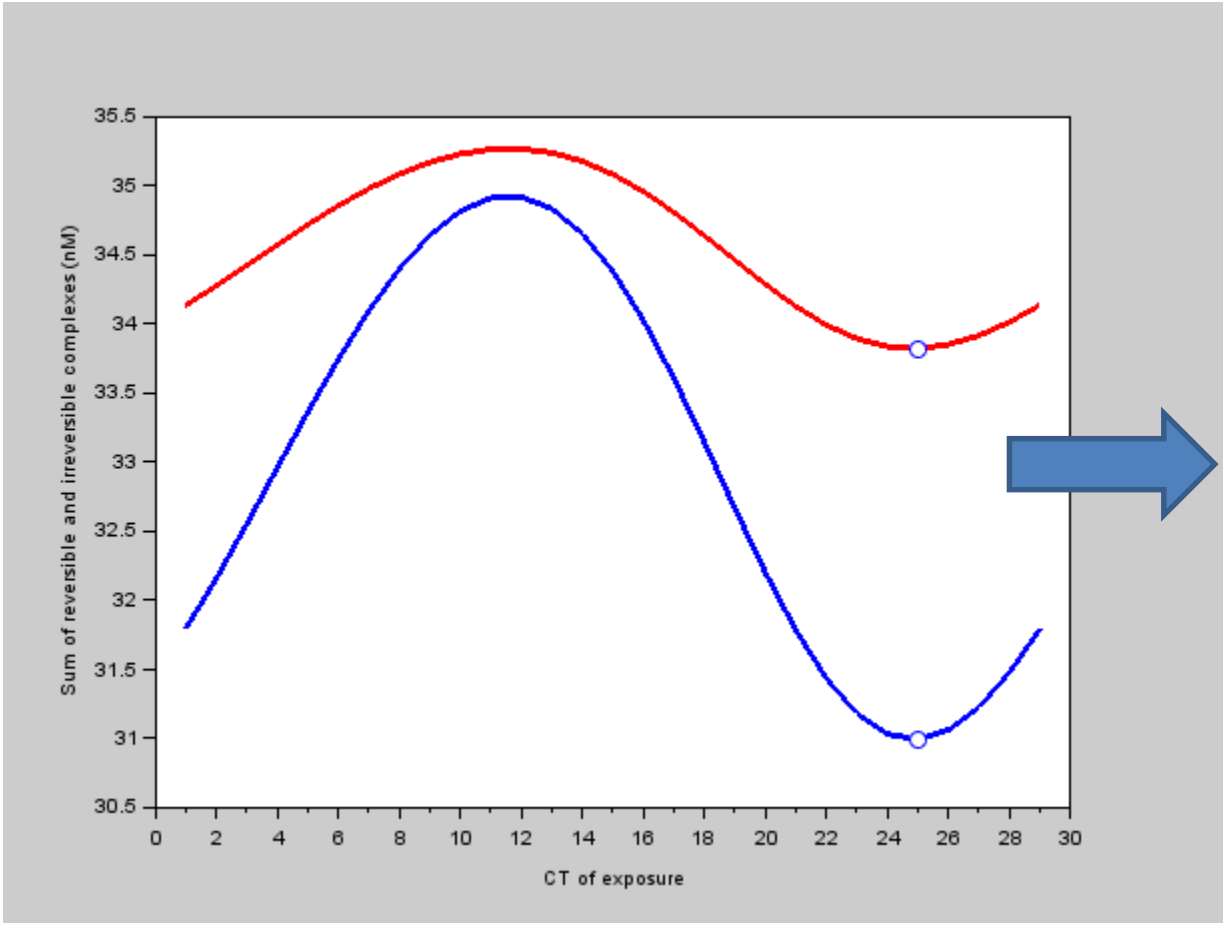


Scilab Tutorial

Exercise 3: Patient Control VS Patient with UGT1A1 polymorphism leading to a division by 3 of the protein activity.



Increased mean toxicity, decreased amplitude

Scilab Tutorial

Exercise 4: Patient with UGT1A1 polymorphism leading to a division by 3 of the protein activity AND lower CPT11 dose (1.4µM)

Scilab Tutorial

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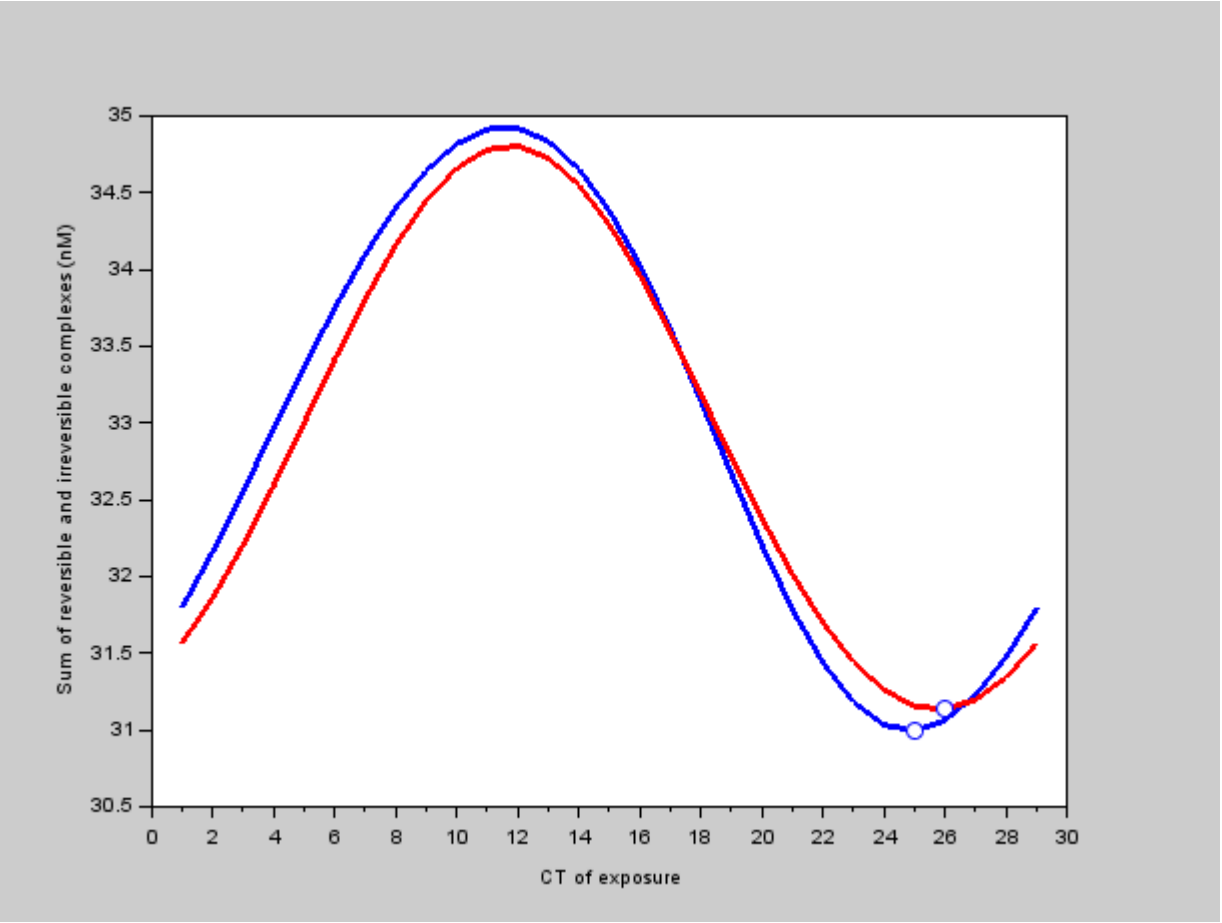

Scilab Tutorial

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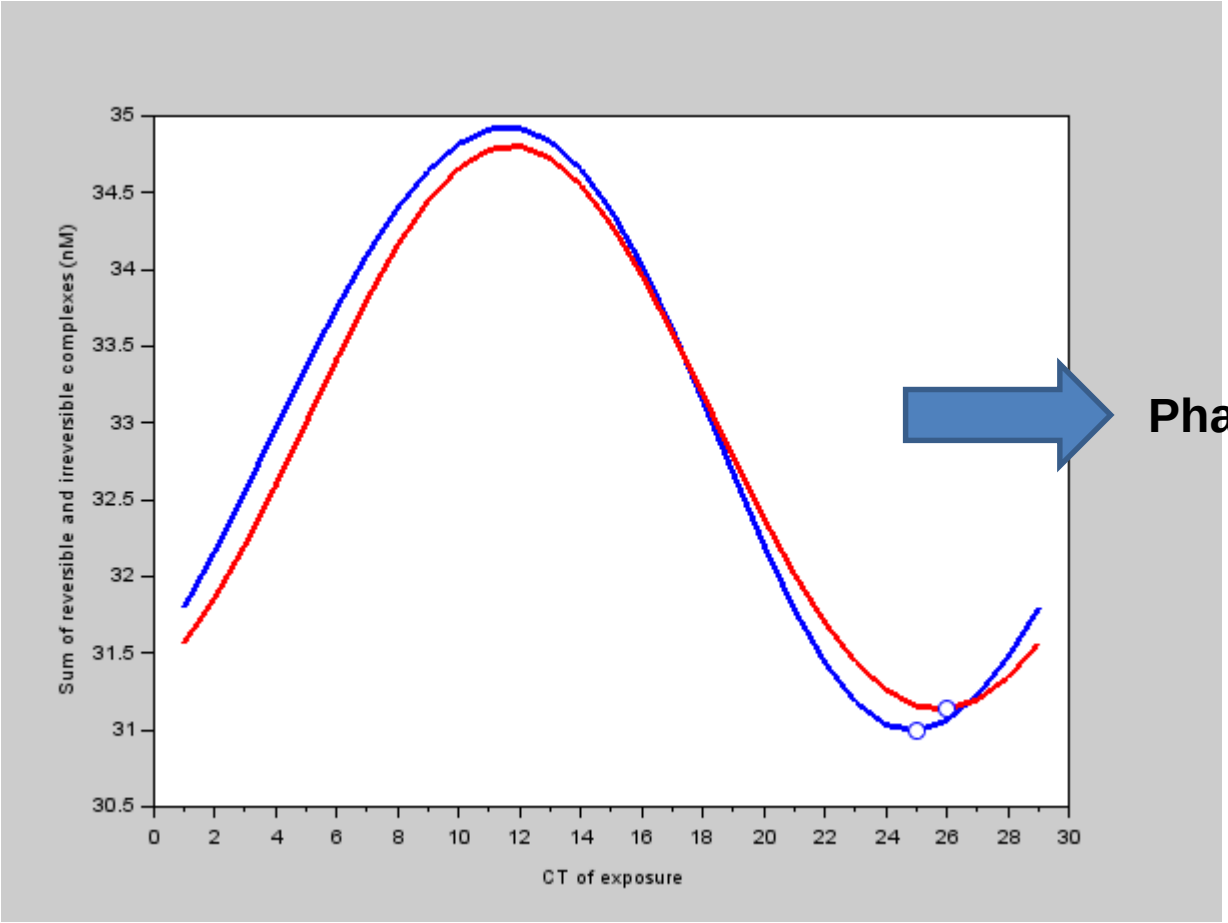

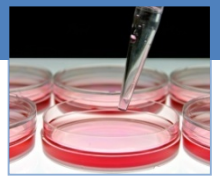

Conclusions of the in vitro study

- **Experimentally-demonstrated circadian organization** in synchronized Caco-2 cell culture

- A **mechanistic mathematical model for CPT11 chronoPK-PD**.

- In vitro systems medicine study demonstrates different chronotoxicity patterns for different circadian and kinetic profiles:
  - Need for personalized chronotherapeutics