# A combined biological and mathematical approach for studying the circadian control of the anticancer drug Irinotecan pharmacokinetics-pharmacodynamics

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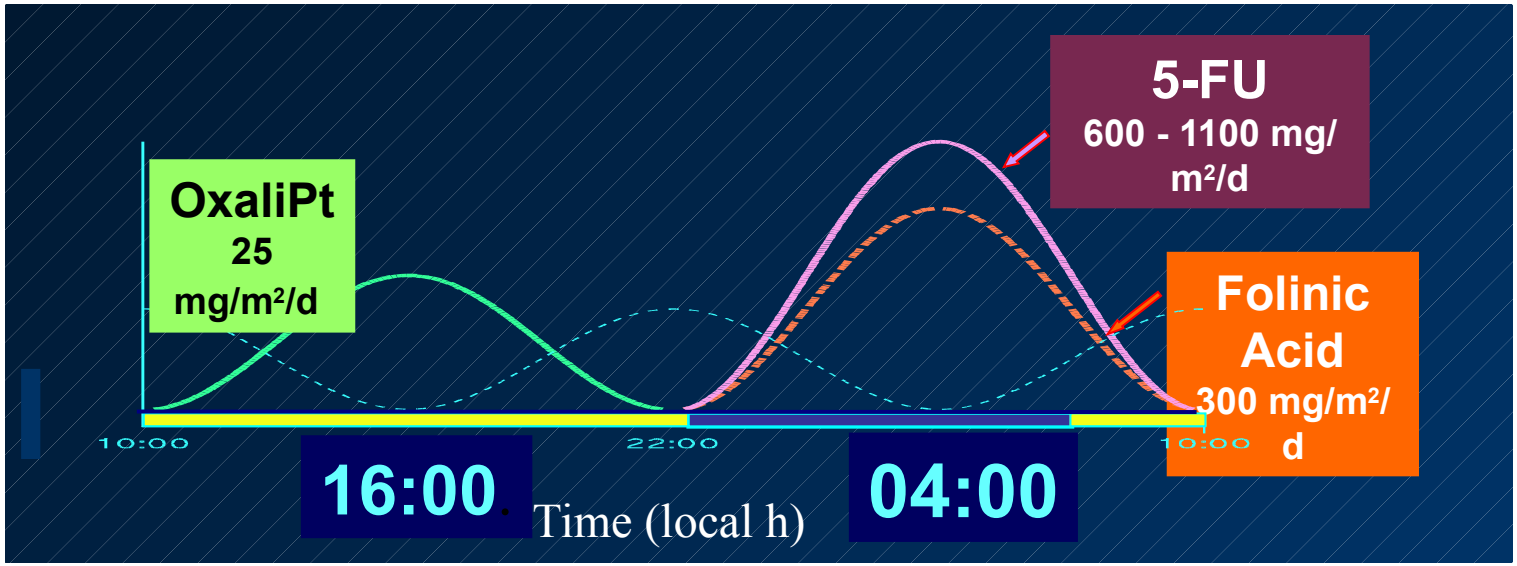
# Introduction: Chronotherapy

- Chronoefficacy/chronotoxicity of many anticancer drugs have been shown in experiments on mice.
- Chronotherapeutic schemes of infusion of the drug have been designed for mice, and then adapted for humans.

Administration Scheme currently used by *Francis Lévi's INSERM team U 776:*




Infusion over 5 days every 3 week

# Introduction: Circadian Rhythms

Results of chronotherapeutics versus constant administration

| Metastatic colorectal cancer (Treated with Folinic Acid, 5-FU, Oxaliplatin) | Infusion flow | |
|---|---|---|
| | CONSTANT | CHRONO |
| Toxicity: | | |
| Oral mucositis gr 3-4 | 74% | 14% |
| Neuropathy gr 2-3 | 31% | 16% |
| Responding rate: | 30% | 51% |

**Chronotherapy improves the responding rate to treatment and decreases the toxicity compared to constant infusion of the drugs.**

# Introduction: Circadian Rhythms

Question:

*Can such drug delivery schedules be improved ?*

# Focus on the anticancer drug Irinotecan

Aims:

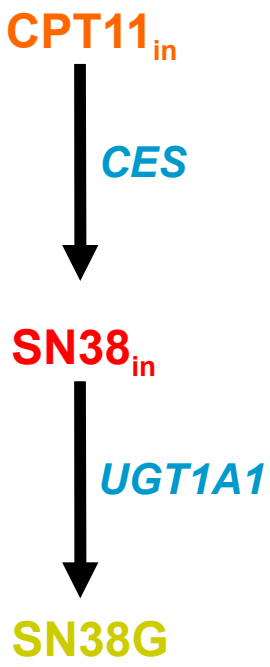
- explain at a molecular level CPT11 chronotoxicity/chronoefficacy.
- find optimal scheme of administration of CPT11, for a given circadian profile.

Means:

1. Cell culture
2. Mathematical Modeling

# 1. Irinotecan Pharmacokinetics/Pharmacodynamics

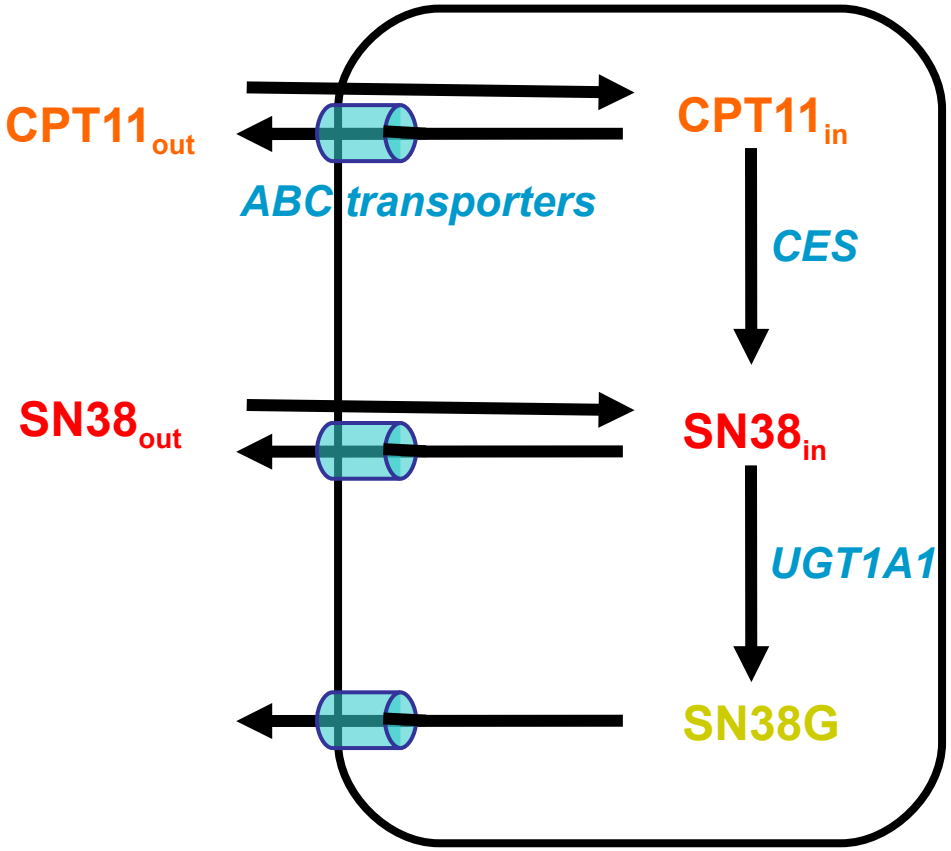
# Irinotecan Pharmacokinetics




Irinotecan(CPT11) is a pro-drug, i.e. it has to be activated into SN-38 which is 1000-fold more efficient . This reaction is catalysed by Carboxylesterases(*CES*).

SN-38 is then glucuronided into SN-38G which is inactive. This reaction is catalysed by the enzyme *UGT1A1*.

# Pharmacokinetics of Irinotecan

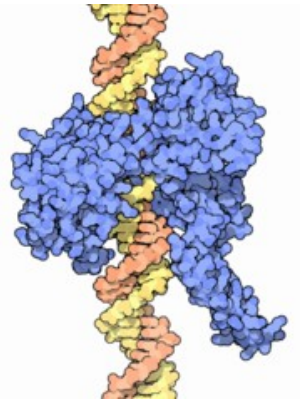
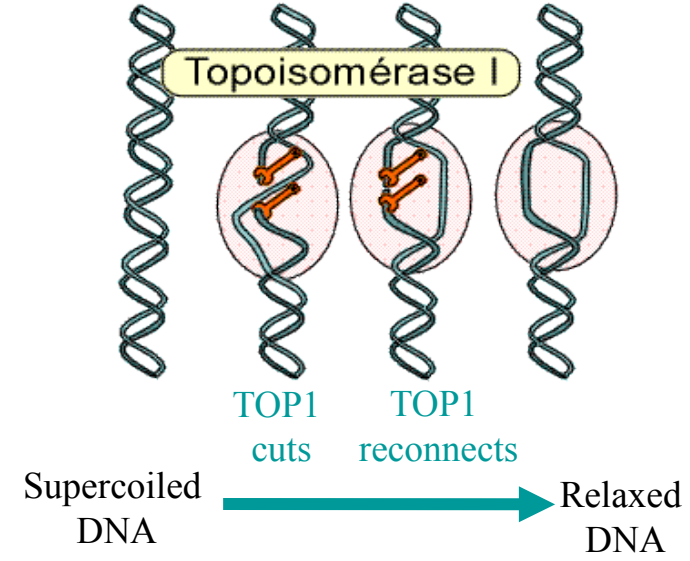



CPT-11, SN-38 and SN-38G are transported outside of the cell by ATP-Binding Cassette (ABC) transporters, which are active efflux pumps.

# Irinotecan Pharmacodynamics

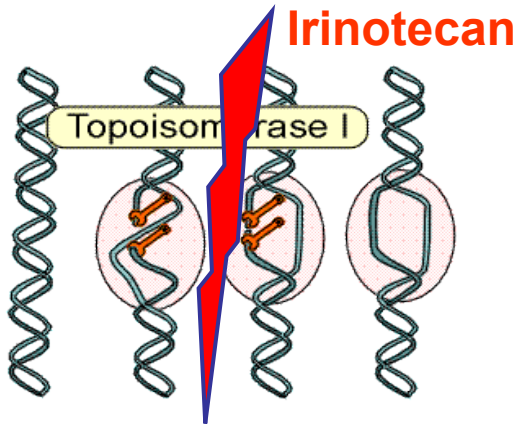
Irinotecan is an **inhibitor of Topoisomerase 1**. What is TOP1?

TOP1 is a nuclear enzyme which is present in heathy cells and aims at relaxing the supercoiled DNA:

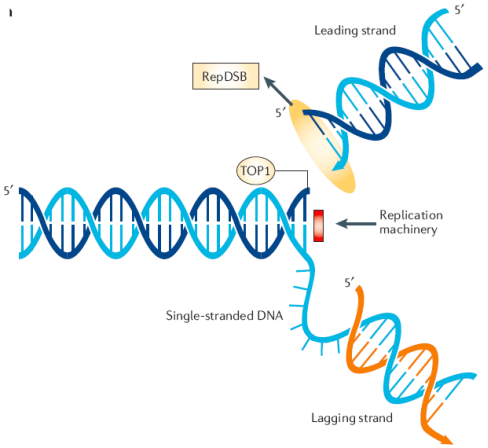
| 1) TOP1 Wraps the supercoiled DNA: | 2) TOP1 cuts one DNA strand so that it can rotate<br>3) TOP1 reconnects the broken strand. |
|---|---|
| TOP1<br>DNA<br>  |  |

# Irinotecan Pharmacodynamics

Irinotecan is an **inhibitor of TOP1**:

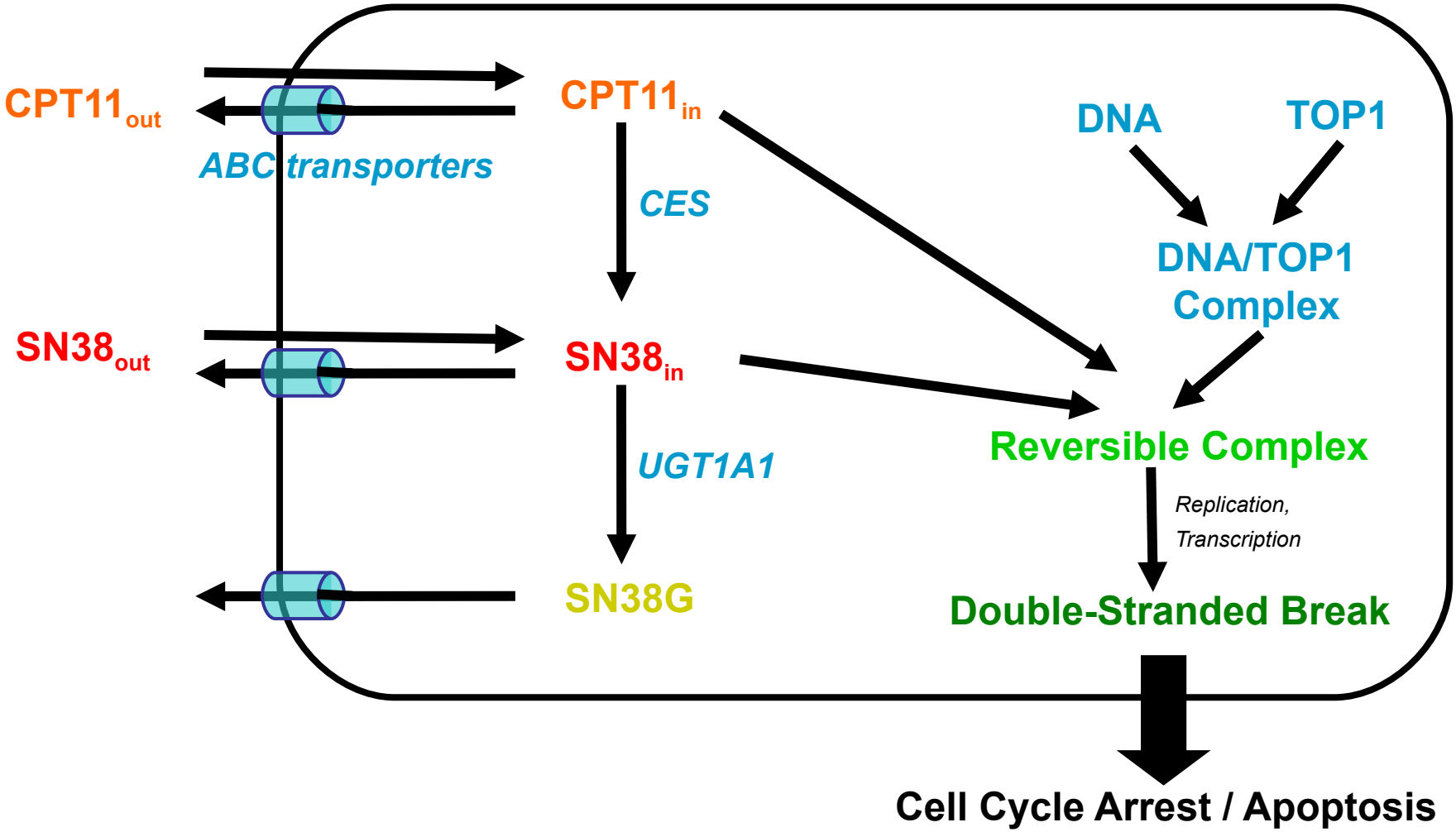



- Irinotecan prevents TOP1 from reconnecting the DNA broken strand, creating reversible TOP1/DNA/Irinotecan complexes.




- The collision between those complexes and replication forks or transcription mechanisms creates DNA double-stranded breaks, which can be lethal for the cell.

# Summary

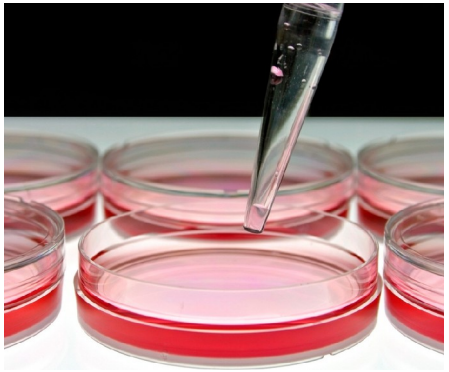

CPT11out
ABC transporters
CPT11in
CES
DNA
TOP1
DNA/TOP1 Complex
SN38out
SN38in
UGT1A1
Reversible Complex
Replication, Transcription
SN38G
Double-Stranded Break
Cell Cycle Arrest / Apoptosis

# 1. Studying Irinotecan in cell culture

# Cell Culture

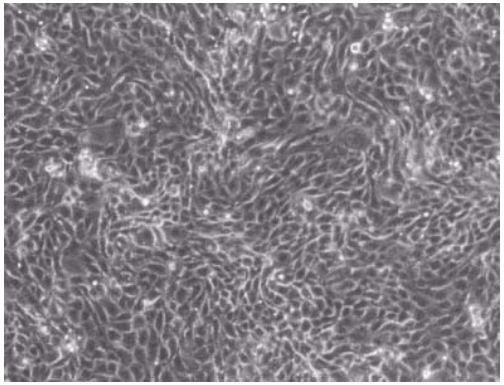
Experiments on Caco-2 cells (human epithelial colorectal adenocarcinoma cells) have been performed.

A Petri Dish



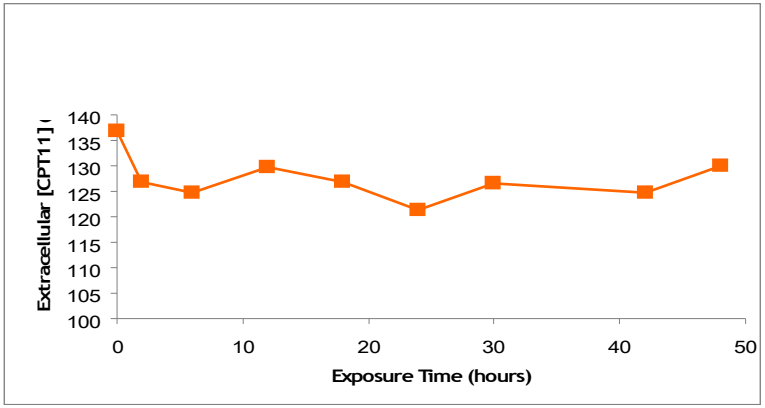
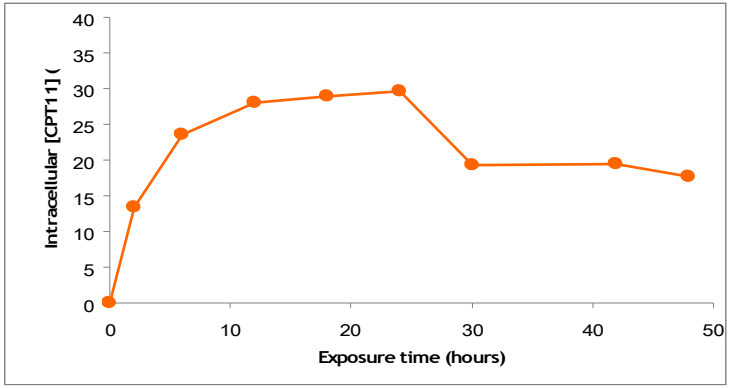
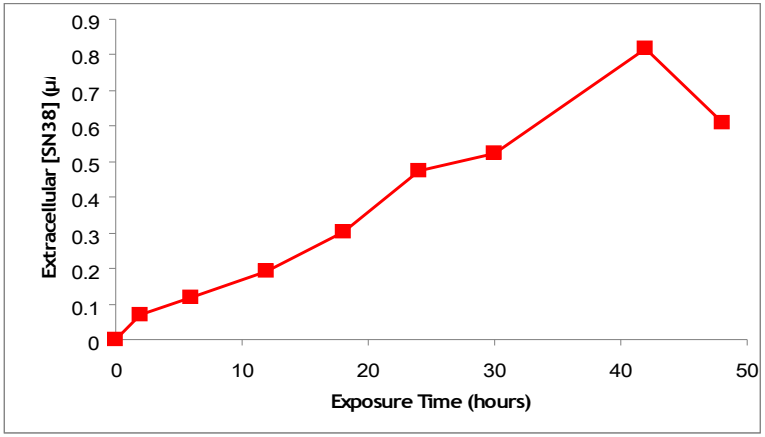
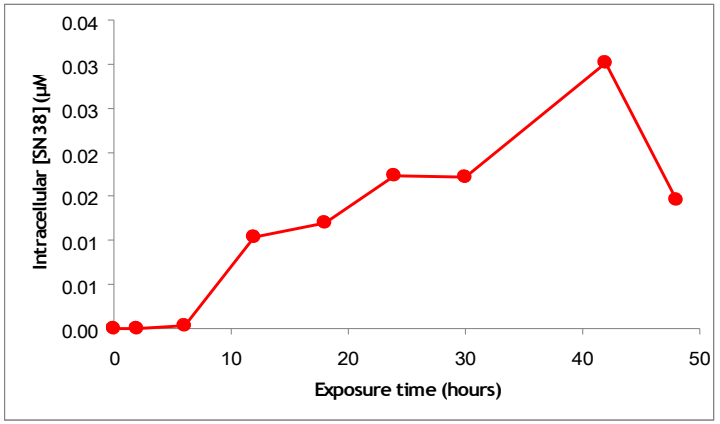
The cells stick to the bottom of the dishes.

The extracellular medium is added on top of the cells



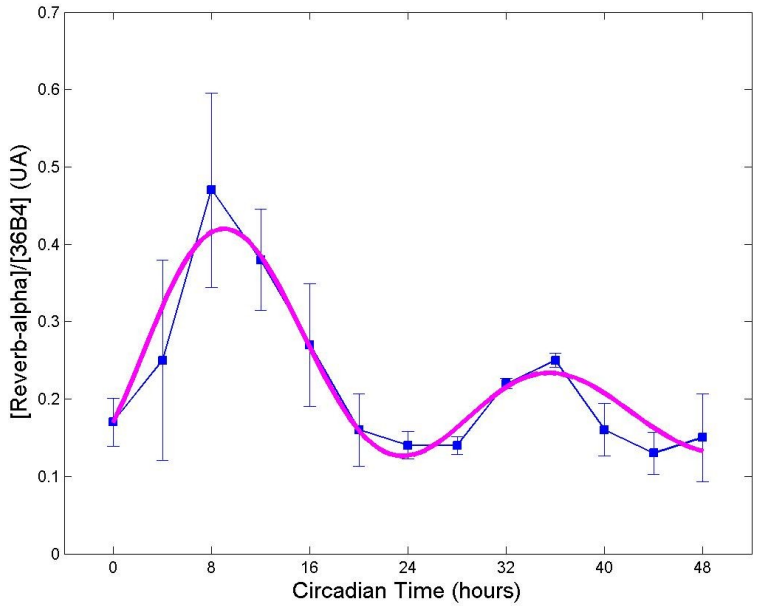
Caco-2 cells under microscope

# Exposure of Caco2 cells to CPT11 (140µM) during 48H
## Measurement of [CPT11] and [SN38] by HPLC

| | Extracellular concentration | Intracellular concentration |
|---|---|---|
| CPT11 |  |  |
| SN38 |  |  |

# Experimental results on Caco-2 cells

- 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.
- Circadian clock oscillate in Caco-2 cells:

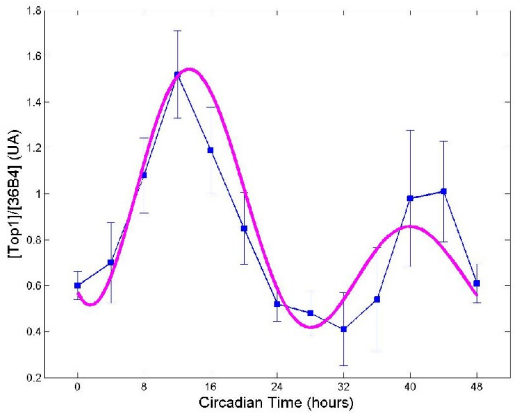



## mRNA Curve Fitting:

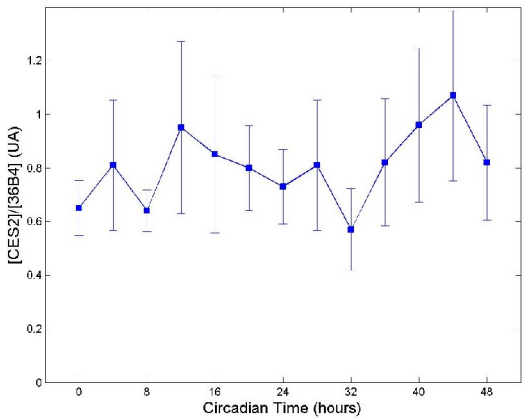
$$[mRNA](t) = R + Se^{\lambda t}(1 + \epsilon cos(\frac{2\pi}{T} + \phi))$$

# Experimental results on Caco-2 cells

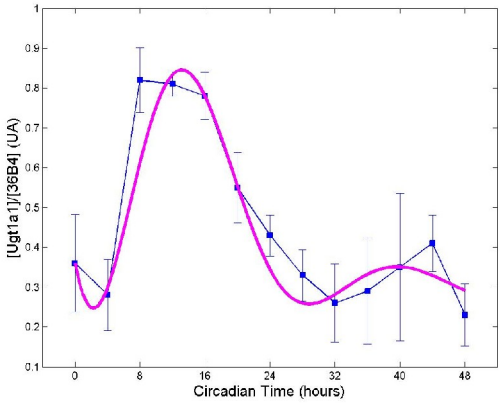
## Topoisomerase 1 (Drug Target)



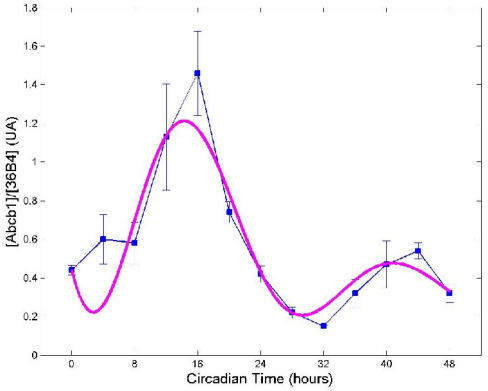

## CES2 (Activation Enzyme)




## UGT1A1 (Deactivation Enzyme )



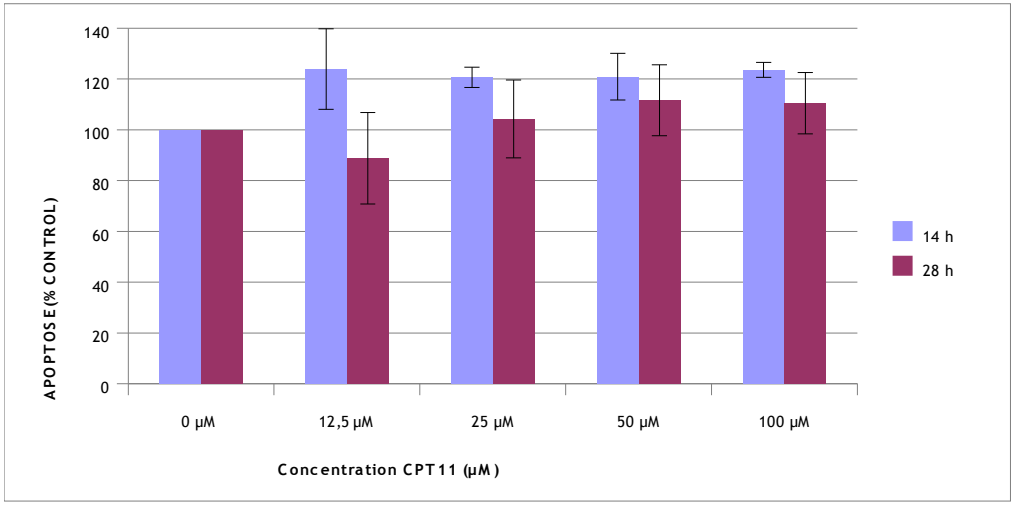

## ABCB1 Transporter



# Experimental results on Caco-2 cells

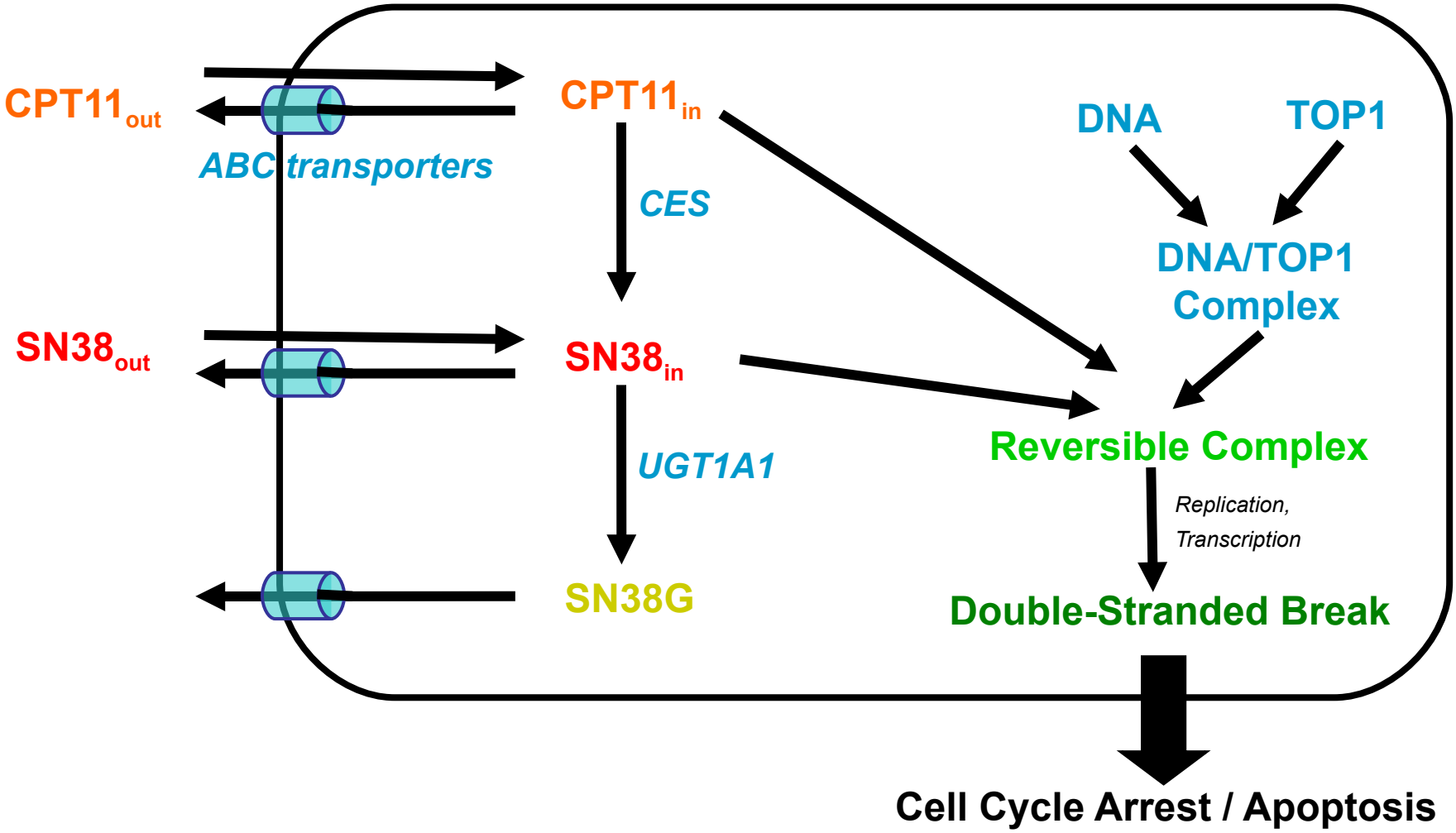
## Irinotecan Chronoefficacy

## Difference in Apoptosis three days after one-hour exposition



# 3. A Mathematical Model including Circadian Rhythms

# Mathematical Modeling



# Mathematical Modeling

- One differential equation for each variable.

- Equation for $[CPT11_{out}]$:

$$\frac{d[CPT11_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{uptakeCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] + \frac{V_{effCPT}[ABC][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]}$$

Change over time

CPT11 cell uptake (passive)

CPT11 cell efflux (active ABC transporters)

$[CPT11_{out}]$ = CPT11 extracellular concentration

$[CPT11_{in}]$ =CPT11 intracellular concentration

$V_{out}$ = volume of extracellular medium

$V_{in}$ =volume of intracellular medium

$k_{uptCPT}$= speed of CPT uptake

$V_{effCPT}$,$K_{eff}$ =Michaelis Menten parameters for CPT efflux

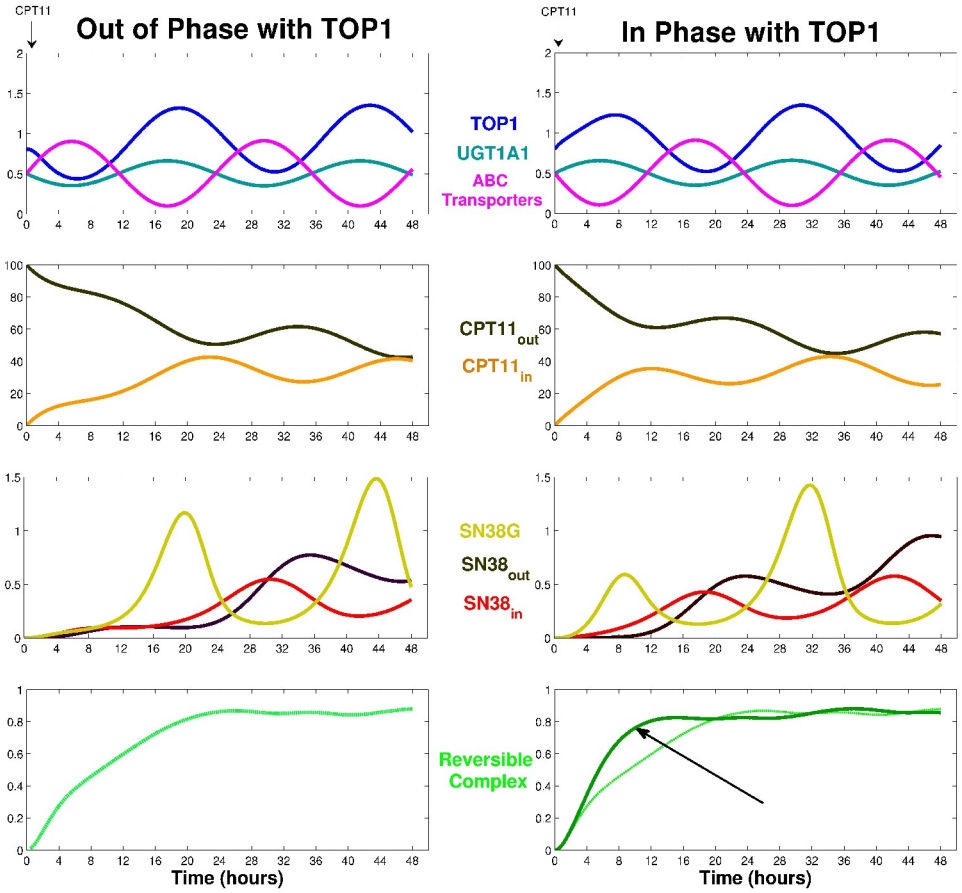
# Mathematical Modeling

## Complete PK-PD model:

$$
\begin{aligned}
\frac{d[CPT11_{out}]}{dt}\frac{V_{out}}{V_{in}} &= -k_{uptakeCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] + \frac{V_{effCPT}[ABC][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]} \\
\frac{d[CPT11_{in}]}{dt} &= k_{uptakeCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] - \frac{V_{effCPT}[ABC][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]} - \frac{V_{CPT-SN}[CPT11_{in}]}{K_{CPT-SN}+[CPT11_{in}]} \\
\frac{d[SN38_{out}]}{dt}\frac{V_{out}}{V_{in}} &= -k_{uptakeSN}\frac{V_{out}}{V_{in}}[SN38_{out}] + \frac{V_{effSN}[ABC][SN38_{in}]}{K_{effSN}+[SN38_{in}]} \\
\frac{d[SN38_{in}]}{dt} &= k_{uptakeSN}\frac{V_{out}}{V_{in}}[SN38_{out}] - \frac{V_{effSN}[ABC][SN38_{in}]}{K_{effSN}+[SN38_{in}]} + \frac{V_{CPT-SN}[CPT11_{in}]}{K_{CPT-SN}+[CPT11_{in}]} \\
&\quad - \frac{V_{SN-SNG}[UGT][SN38_{in}]}{K_{SN-SNG}+[SN38_{in}]} - kf_C[TOP1][SN38_{in}](DNA_{tot}-[COMPL]) + kr_C[COMPL] \\
\frac{d[SN38G]}{dt} &= \frac{V_{SN-SNG}[UGT][SN38_{in}]}{K_{SN-SNG}+[SN38_{in}]} - \frac{V_{effSNG}[ABC][SN38G]}{K_{effSNG}+[SN38G]} \\
\frac{d[COMPL]}{dt} &= kf_C[TOP1][SN38_{in}](DNA_{tot}-[COMPL]) - kr_C[COMPL]
\end{aligned}
$$

# Mathematical Modeling

## Simulation: chosing the right circadian time to expose cells



# Conclusion and future work

- More data are expected (CPT-11, SN-38, SN-38G transport kinetics, reversible complexes formation/dissociation, protein level…)
- Once the mathematical model is calibrated and validated (by other cell culture experiments), it will be use to define a **theoritically optimal scheme** for exposition of Caco-2 cells to Irinotecan.
- Future: this study at the cell population level may then be integrated into a whole-body approach (modeling tissular CPT11 PK-PD) for the mouse.

# Future: Whole Body Mathematical Model

Aim: design theoretically optimal scheme of administration for the three mouse chronotoxicity classes (cf. C. Ahowesso work).

Mean: Whole Body PK PD Model based on the mathematical model of cell culture (cf. H. Gayrard work)