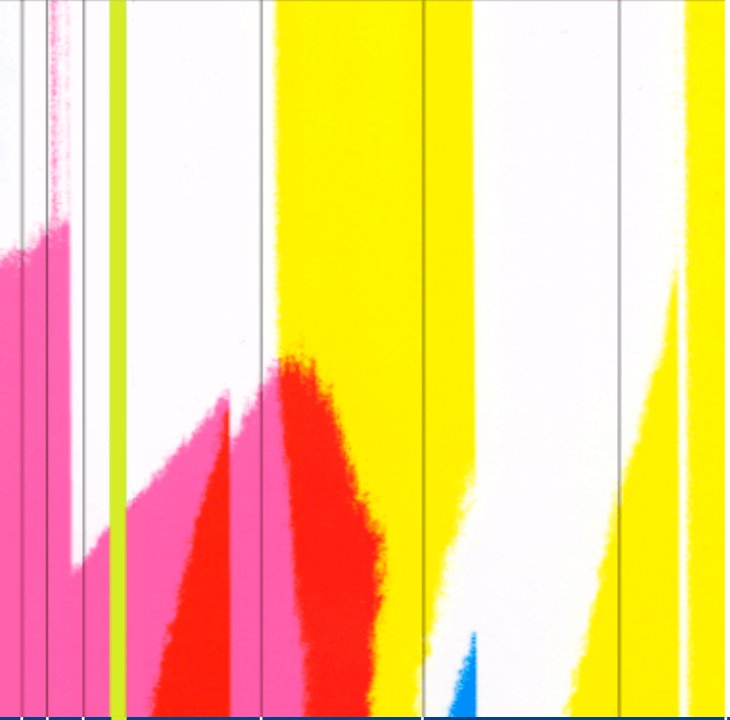

A Combined Biological and Mathematical Approach for Modeling PK-PD of Anticancer Drug Irinotecan -

Focus on Acquired Resistance and Circadian Rhythms at the Cell Population Scale

Annabelle Ballesta[1], Jean Clairambault[1], Sandrine Dulong[2], Alper Okyar[2], Francis Levi[2]

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INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE

INRIA

centre de recherche PARIS - ROCQUENCOURT

[1] *BANG Project, INRIA- Rocquencourt*
[2] *U776 "Rythmes Biologiques et Cancer", INSERM - Villejuif*

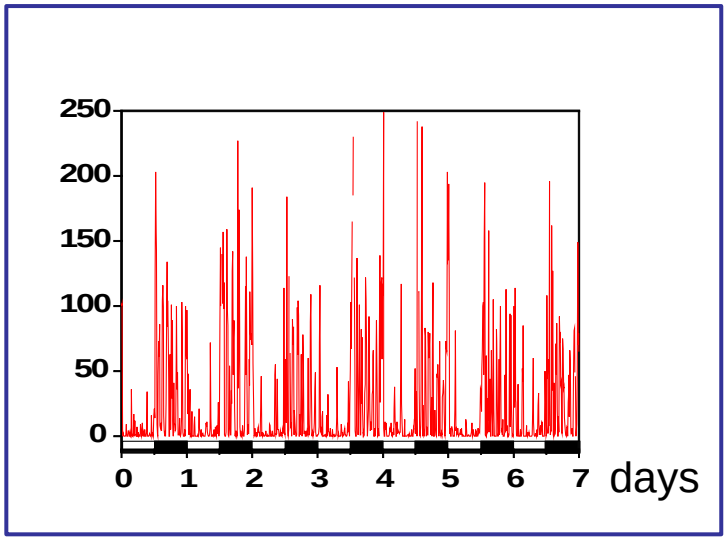
Outline

1. Irinotecan Pharmacokinetics/Pharmacodynamics
2. Studying Irinotecan in cell culture
3. Decrease in Intracellular Concentration: Acquired Resistance ?
    1. Experimental Results
    2. Mathematical Modeling
4. An Extended Model including Circadian Rhythms
    1. Experimental Results on Circadian Rhythms
    2. Mathematical Modeling

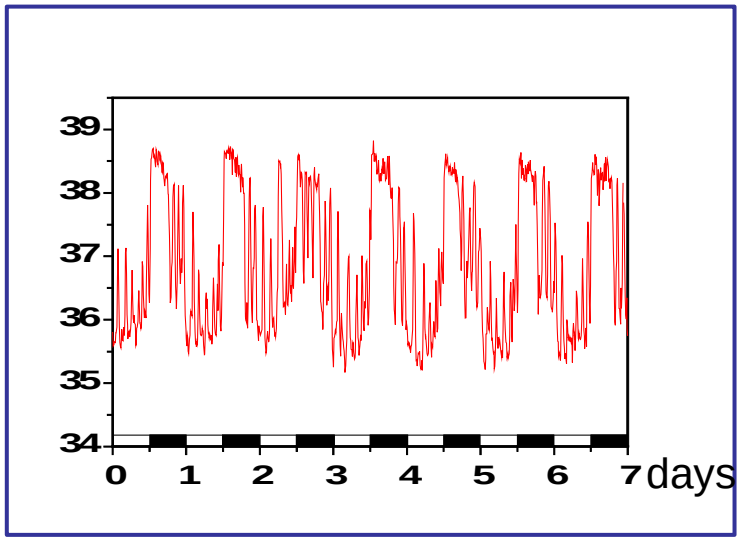
Introduction: Circadian Rhythms

- Circadian = around 24 hours.

- Example of the circadian rhythm in mice:



Rest-activity rhythm in mice

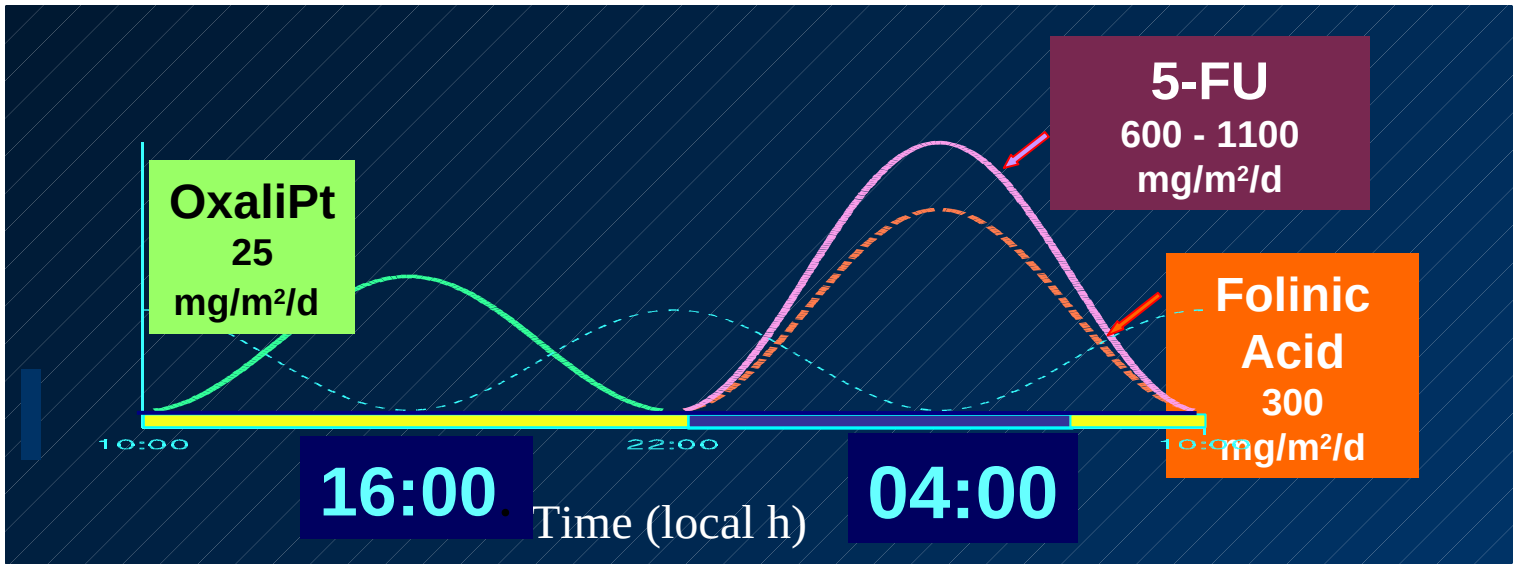


Body temperature in mice

Introduction: Circadian Rhythms

Time-scheduled delivery regimen for Metastatic Colorectal Cancer

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



Infusion over 5 days every 3 week

Chronotherapeutic schemes of infusion of the drug have been designed for the mouse, and then adapted for the human.

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 ?

1. Irinotecan Pharmacokinetics/Pharmacodynamics

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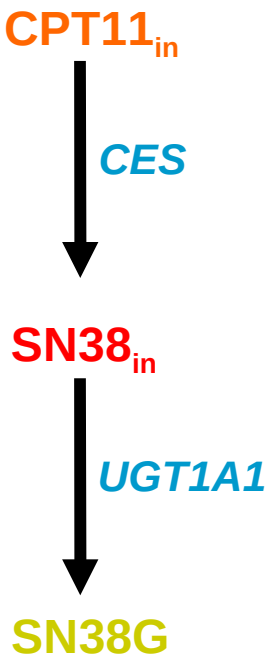
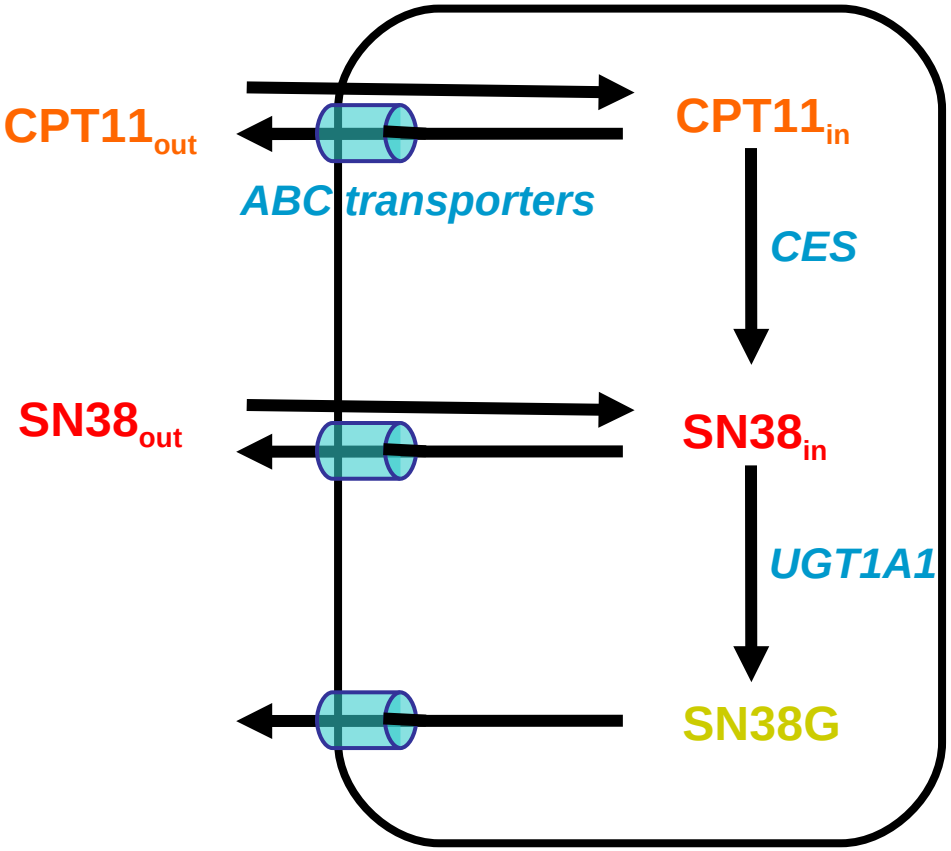
Pharmacokinetics of Irinotecan



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



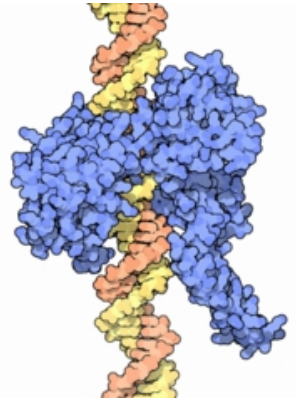
CPT-11, SN-38 and SN-38G are transported outside of the cell by ATP-Binding Cassette (ABC) transporters, which are active efflux pumps.

Pharmacodynamics of Irinotecan

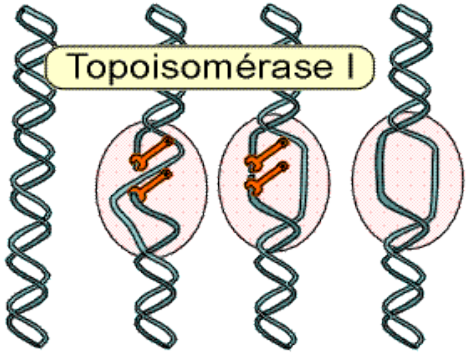
Irinotecan is an **inhibitor of Topoisomerase I**

The Topoisomerase I is an enzyme that:

- Wraps the supercoiled DNA :



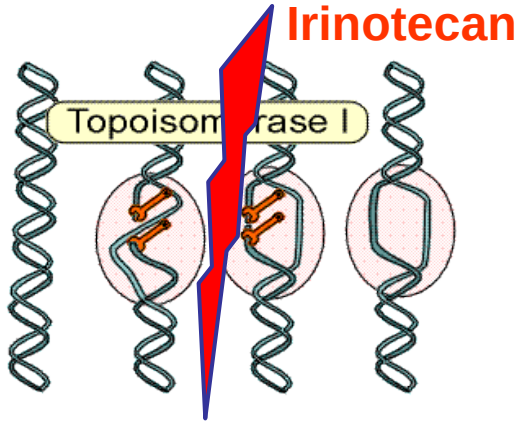
- Cuts one strand so that the DNA can relax
- Reconnects broken strands



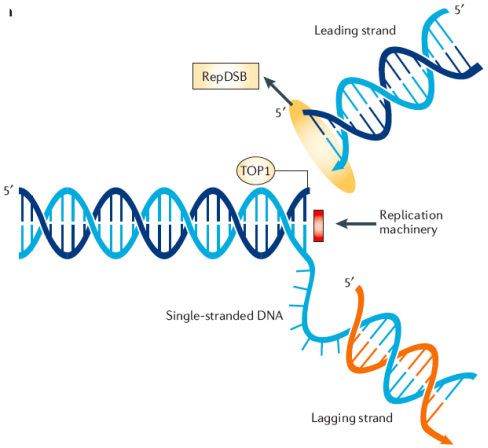
Pharmacodynamics of Irinotecan

Irinotecan is an **inhibitor of TOP1**:

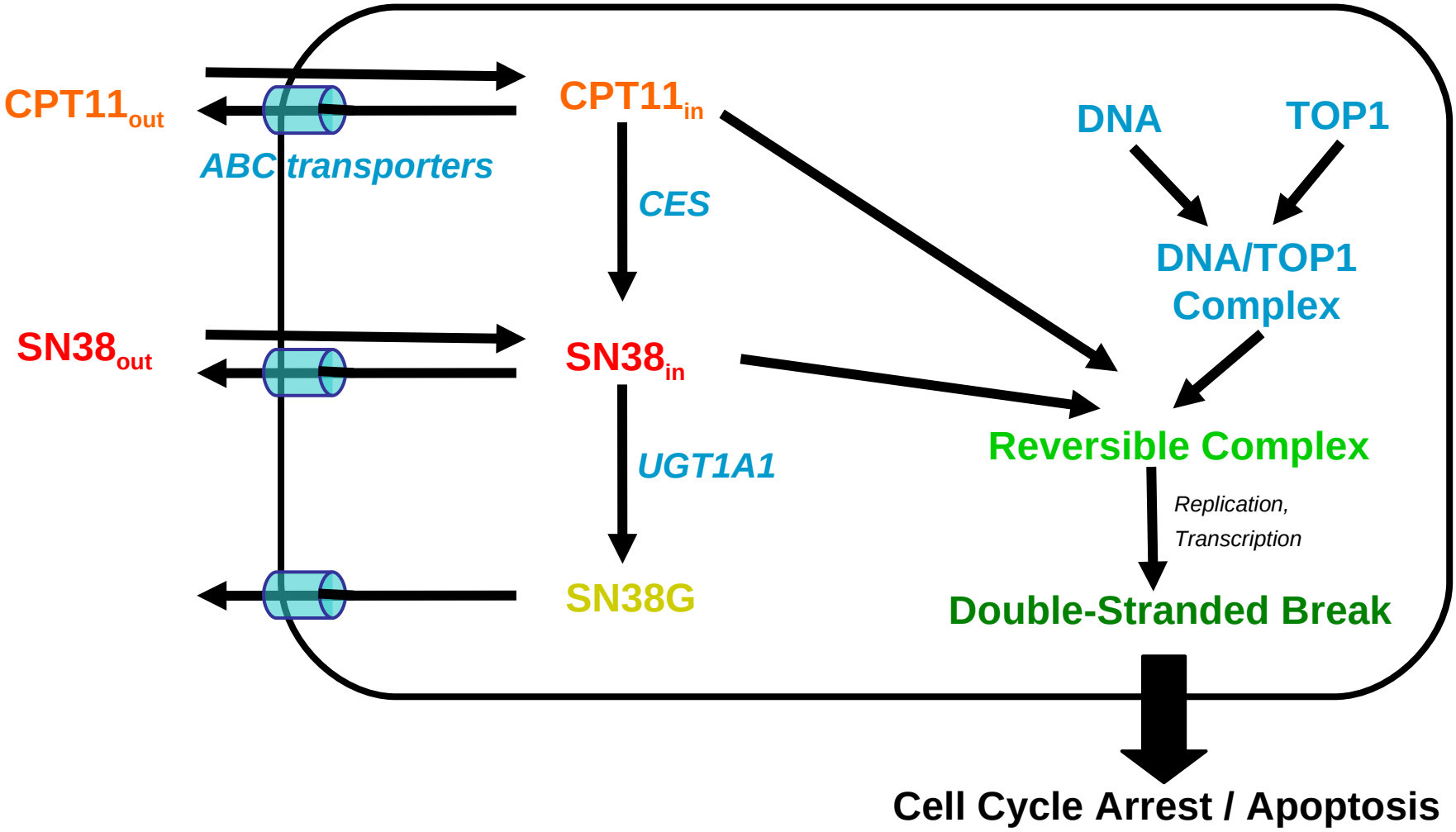
- Irinotecan prevents TOP1 from reconnecting the broken strands of the DNA, creating reversible ternary complexes TOP1/DNA/Irinotecan.



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



Summary



1. Studying Irinotecan in cell culture

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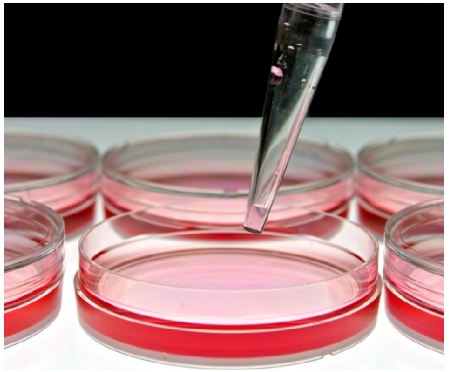
Cell Culture

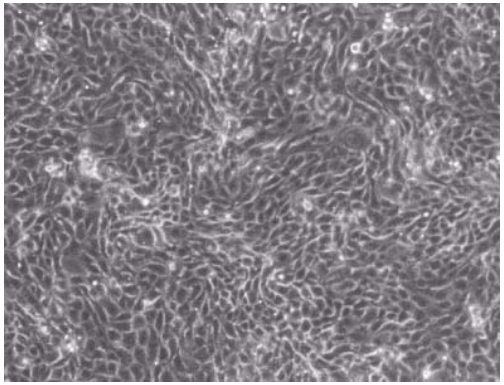
Experiments on Caco-2 cells (human epithelial colorectal adenocarcinoma cells) have been performed.

A Petri Dish



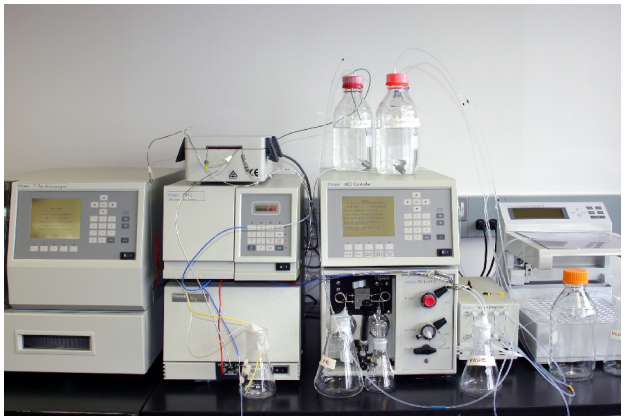
The cells stick to the bottom of the dishes.

The extracellular medium is added on top of the cells



Caco-2 cells under microscope

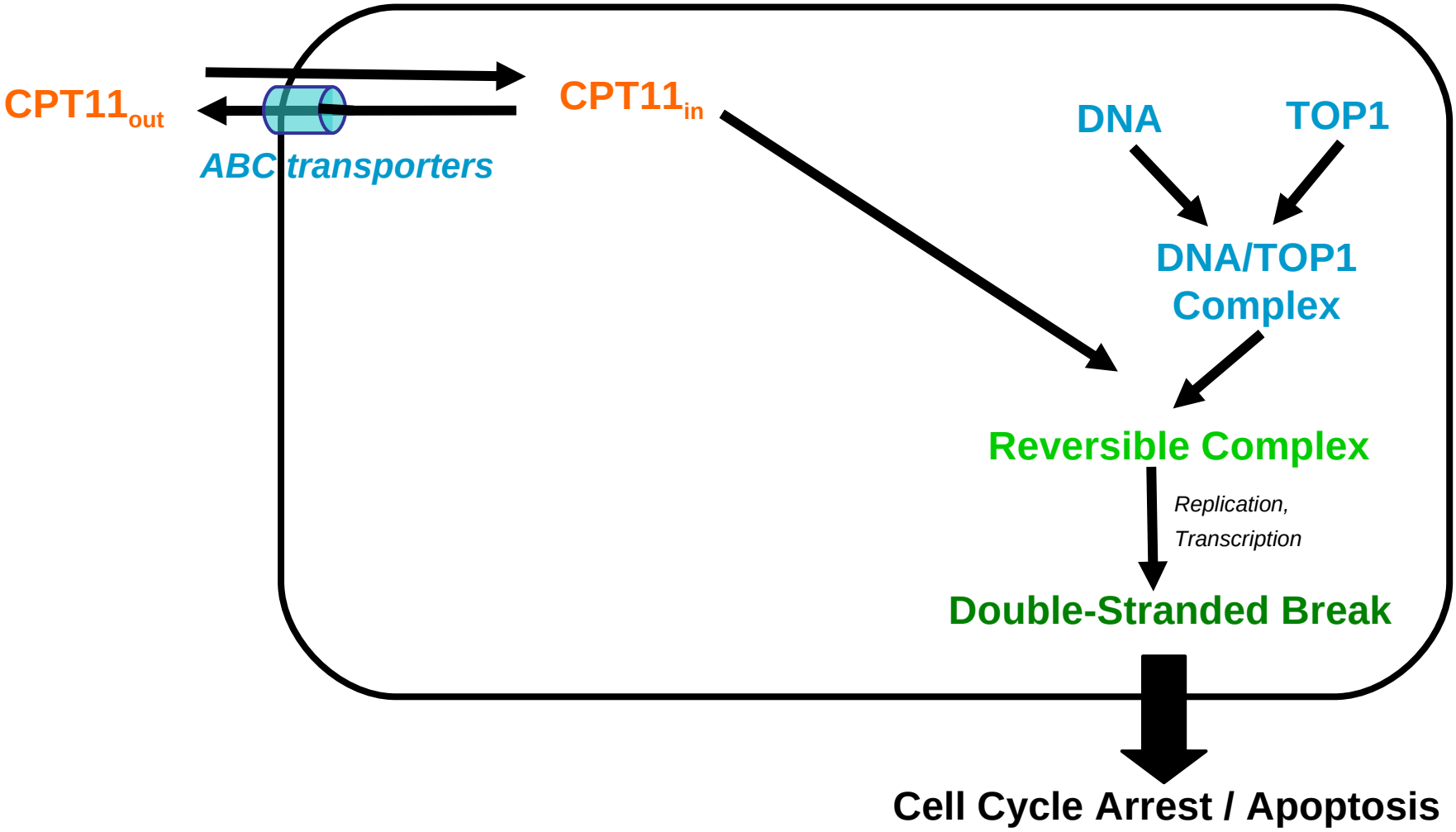
Detection of CPT11 and its metabolite by High Performance Liquid Chromatography(HPLC)



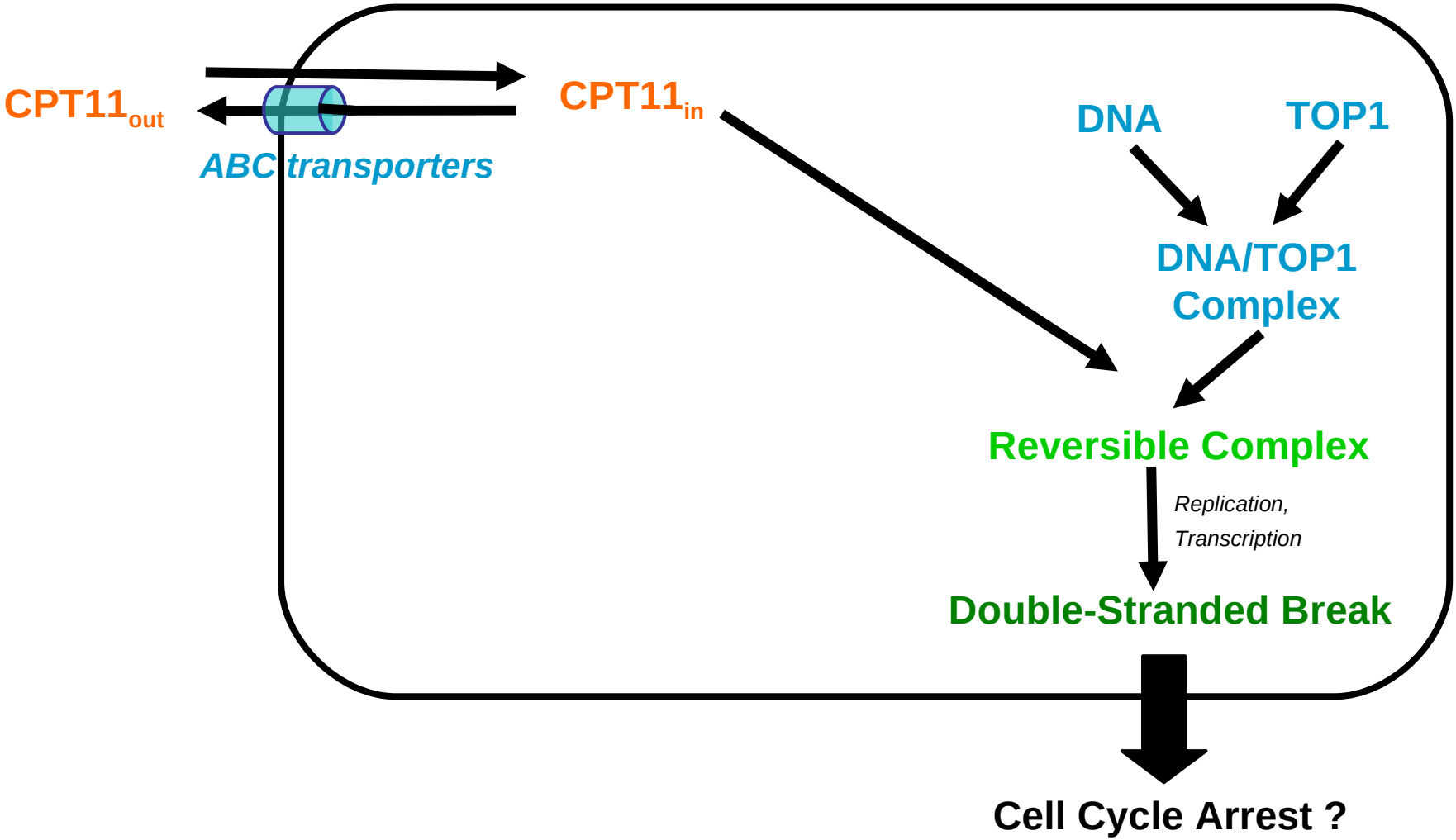
What we theoretically detect:

- CPT11 OUT = CPT11 in the extracellular medium
- CPT11 IN = CPT11 in the intracellular medium+ CPT11 trapped in complexes with Topoisomerase I.
- SN38 OUT = SN38 in the extracellular medium
- SN38 IN = SN38 in the intracellular medium+ SN38 trapped in complexes with Topoisomerase I.

No SN38 is detected by HPLC

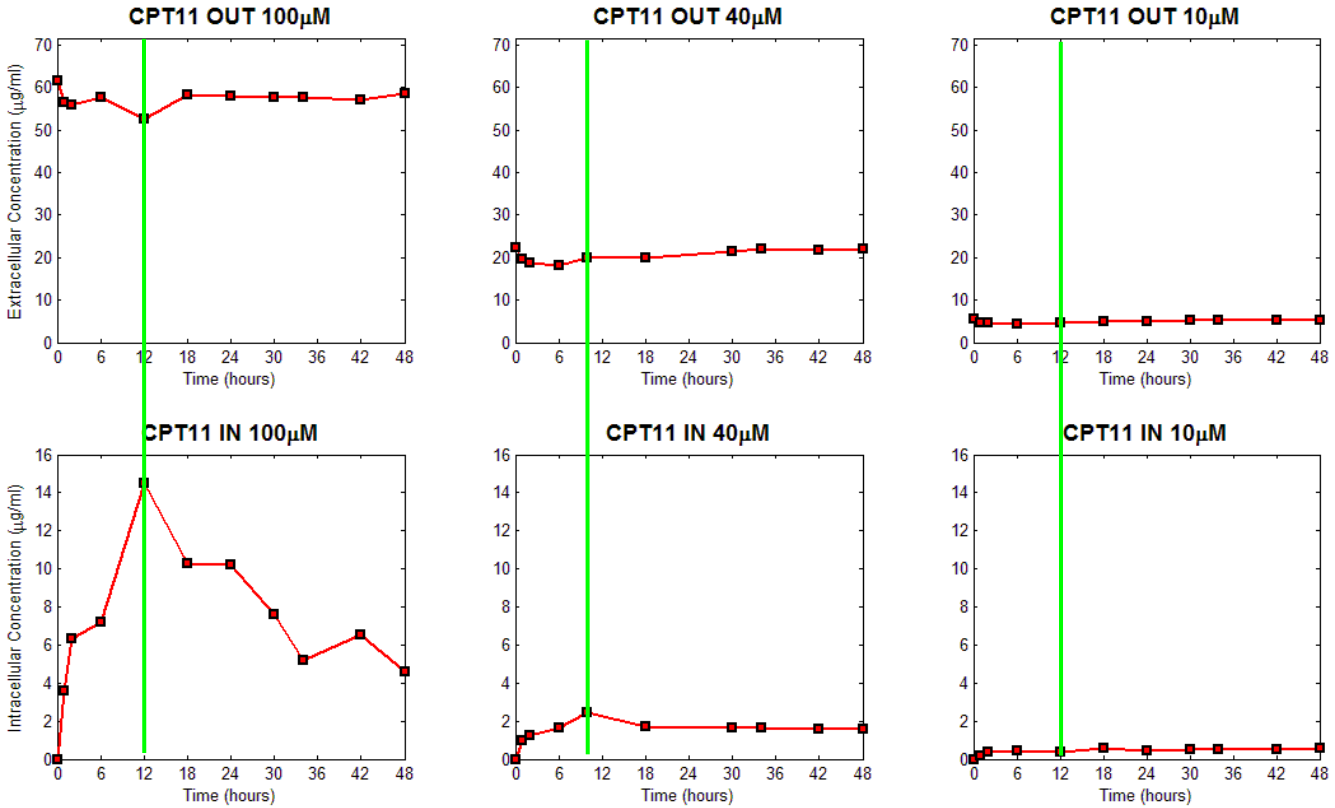


CPT11 is not cytotoxic for Caco-2 cells



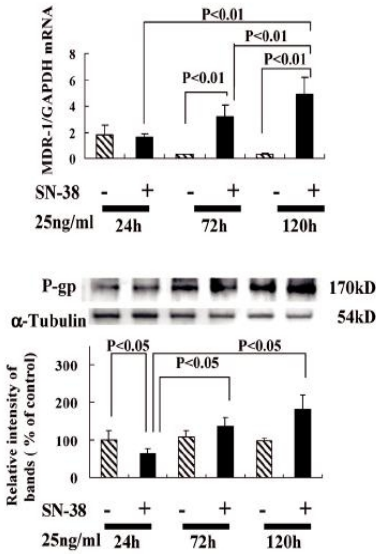
3. Decrease in Intracellular Concentration: Acquired Resistance ?

Experimental results



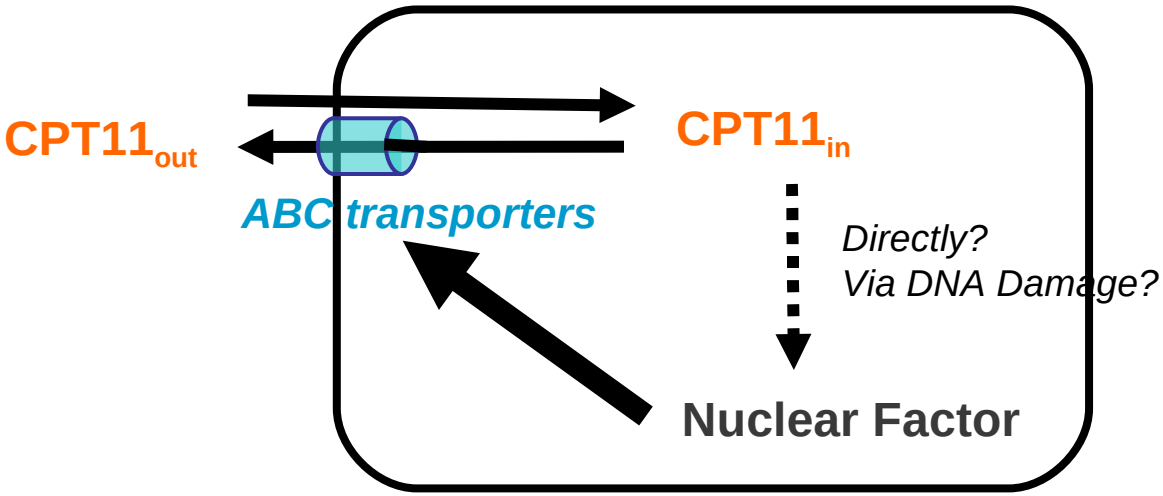
Decrease in Intracellular Concentration

- Possible Explanation for Those Results: Induction of ABC transporters.
- Pgp is inducible by SN38 in HUH7 cells (human hepatocellular carcinoma cells) : cf. Takeba et al., *Irinotecan-Induced Apoptosis Is Inhibited by Increased P-Glycoprotein Expression and Decreased p53 in Human Hepatocellular Carcinoma Cells, Biol. Pharm. Bull.* **30**(8) 1400—1406 (2007)



Decrease in Intracellular Concentration

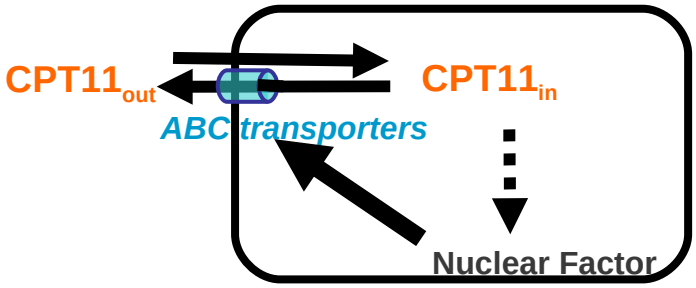
- CPT11 activates the nuclear factor NFkappaB (cf. Bottero et al. *Activation of Nuclear Factor B through the IKK Complex by the Topoisomerase Poisons SN38 and Doxorubicin: A Brake to Apoptosis in HeLa Human Carcinoma Cells,* CANCER RESEARCH 61, 7785–7791, November 1, 2001])
- Pgp is induced by the nuclear factor NFkappaB (cf. Zhou et al. *NF-kB-mediated Induction of mdr1b Expression by Insulin in Rat Hepatoma Cells,* THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 272, No. 24, Issue of June 13, pp. 15174–15183, 1997)
- Proposition of a Model:



Mathematical Modeling

Construction of an ODE-based model:

- One equation for each variable.



- Example: the equation for [$CPT11_{out}$]:

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

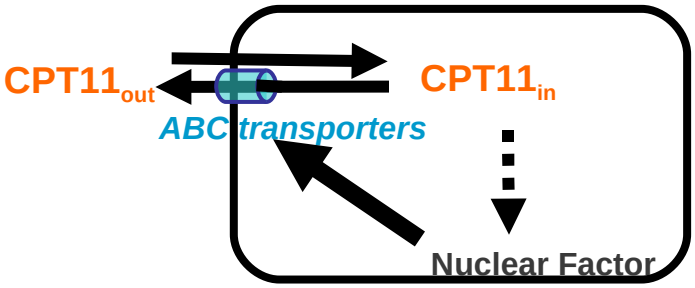
Rate of change over time

Uptake

Efflux

Mathematical Modeling

ODE-based Model:



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

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

$$\frac{d[NF]}{dt} = \frac{[CPT11_{in}]^n}{K_{ind}^n + [CPT11_{in}]^n} - k_{dNF}[NF]$$

$$\frac{d[ABC]}{dt} = k_{fABC} + k_{ind}[NF] - k_{dABC}[ABC]$$

Mathematical Modeling

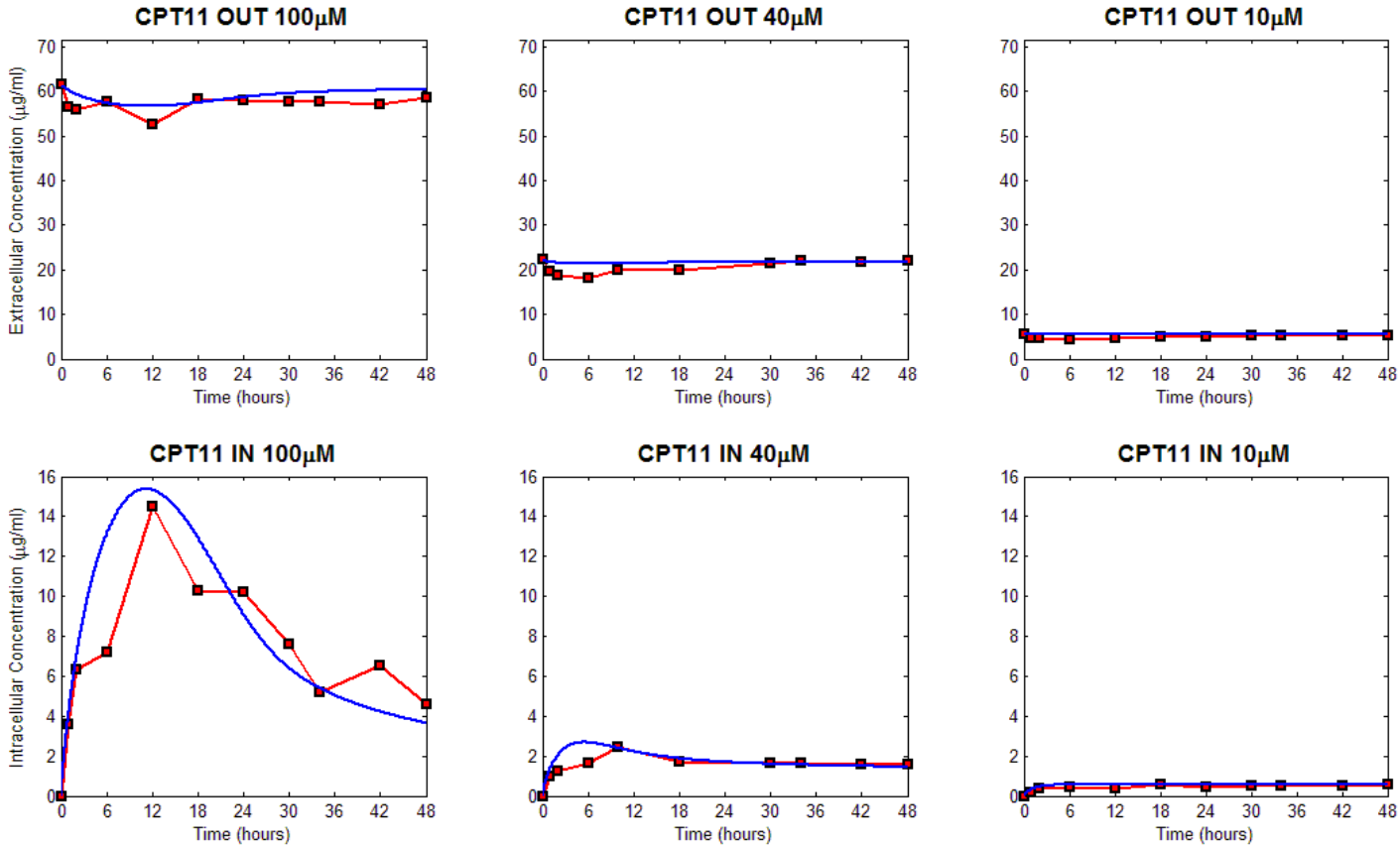
Conservation Law: *the total quantity of CPT11 is conserved*

$$
\begin{aligned}
n_{out} + n_{in} &= n_0 = CPT11_{out}(t=0)V_{out} \\
CPT11_{out}V_{out} + CPT11_{in}V_{in} &= n_0 \\
CPT11_{out} &= \frac{n_0 - CPT11_{in}V_{in}}{V_{out}}
\end{aligned}
$$

System of equation to be solved:

$$
\begin{aligned}
\frac{d[CPT11_{in}]}{dt} &= k_{uptakeCPT}(C_0/V_{in} - CPT11_{in}) - \frac{V_{effCPT}/V_{in}[ABC][CPT11_{in}]}{\frac{K_{effCPT}}{V_{in}} + [CPT11_{in}]} \\
\frac{d[NF]}{dt} &= \frac{[CPT11_{in}]^n}{K_{ind}^n + [CPT11_{in}]^n} - k_{dNF}[NF] \\
\frac{d[ABC]}{dt} &= k_{fABC} + k_{ind}[NF] - k_{dABC}[ABC]
\end{aligned}
$$

Mathematical Modeling



Mathematical Modeling

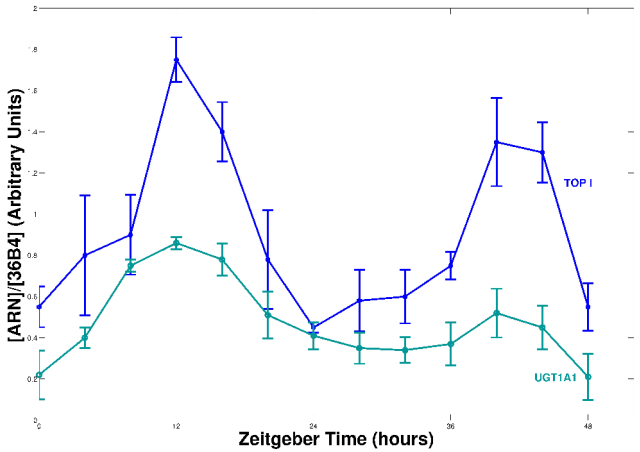
- Conclusion: the suggested model is able to reproduce the experimental data.

- Work in progress to confirm our hypothesis:
  1. Measurements of CPT11 Intracellular/Extracellular concentration with inhibitor of ABC transporters (Verapamil).
  2. Measurements of Pgp mRNA level over time of exposure.

4. An Extended Model including Circadian Rhythms

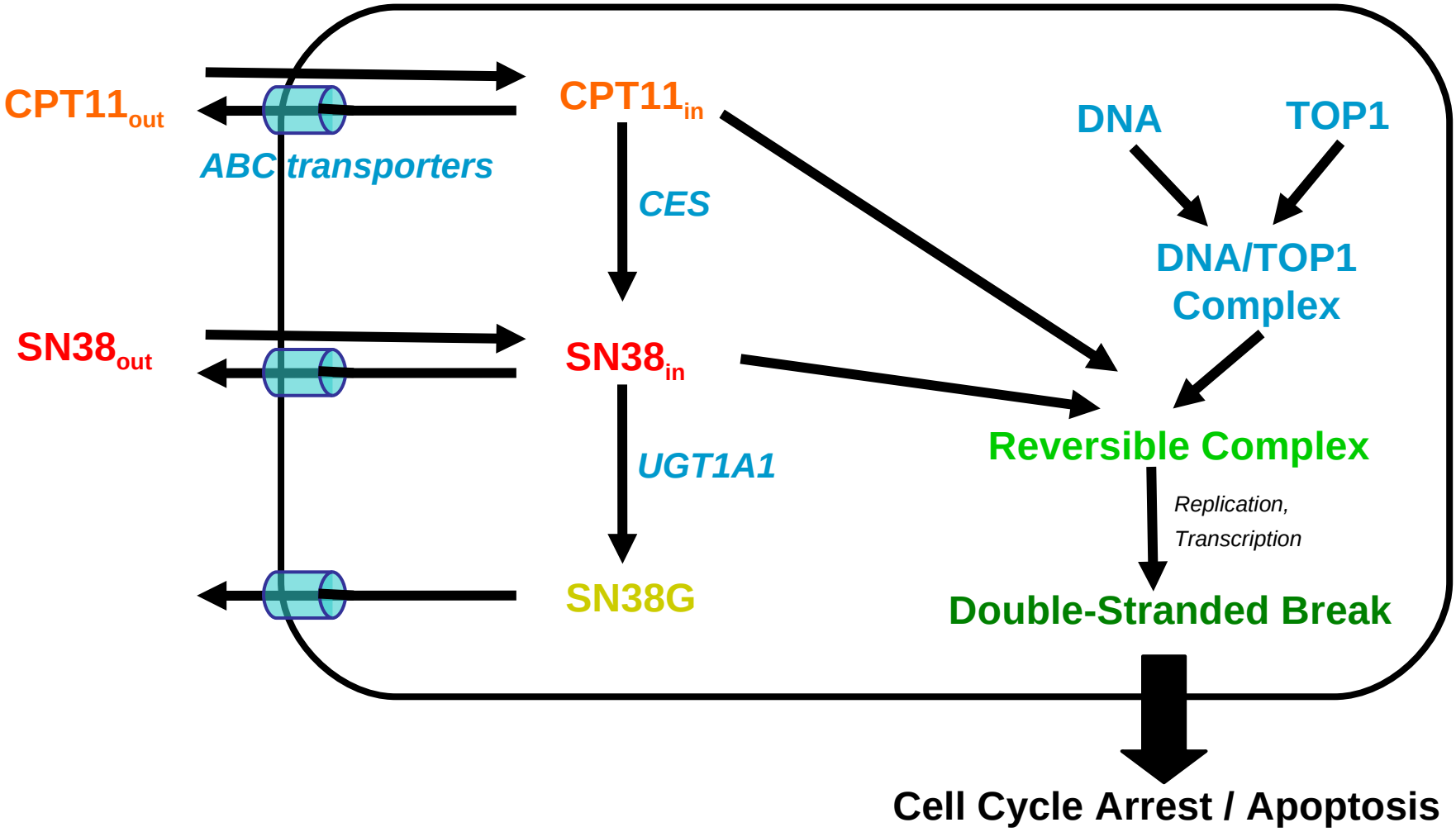
Experimental results on Caco-2 cells

- 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.
- Topoisomerase I and UGT1A1 have circadian rhythms in Caco-2 cells

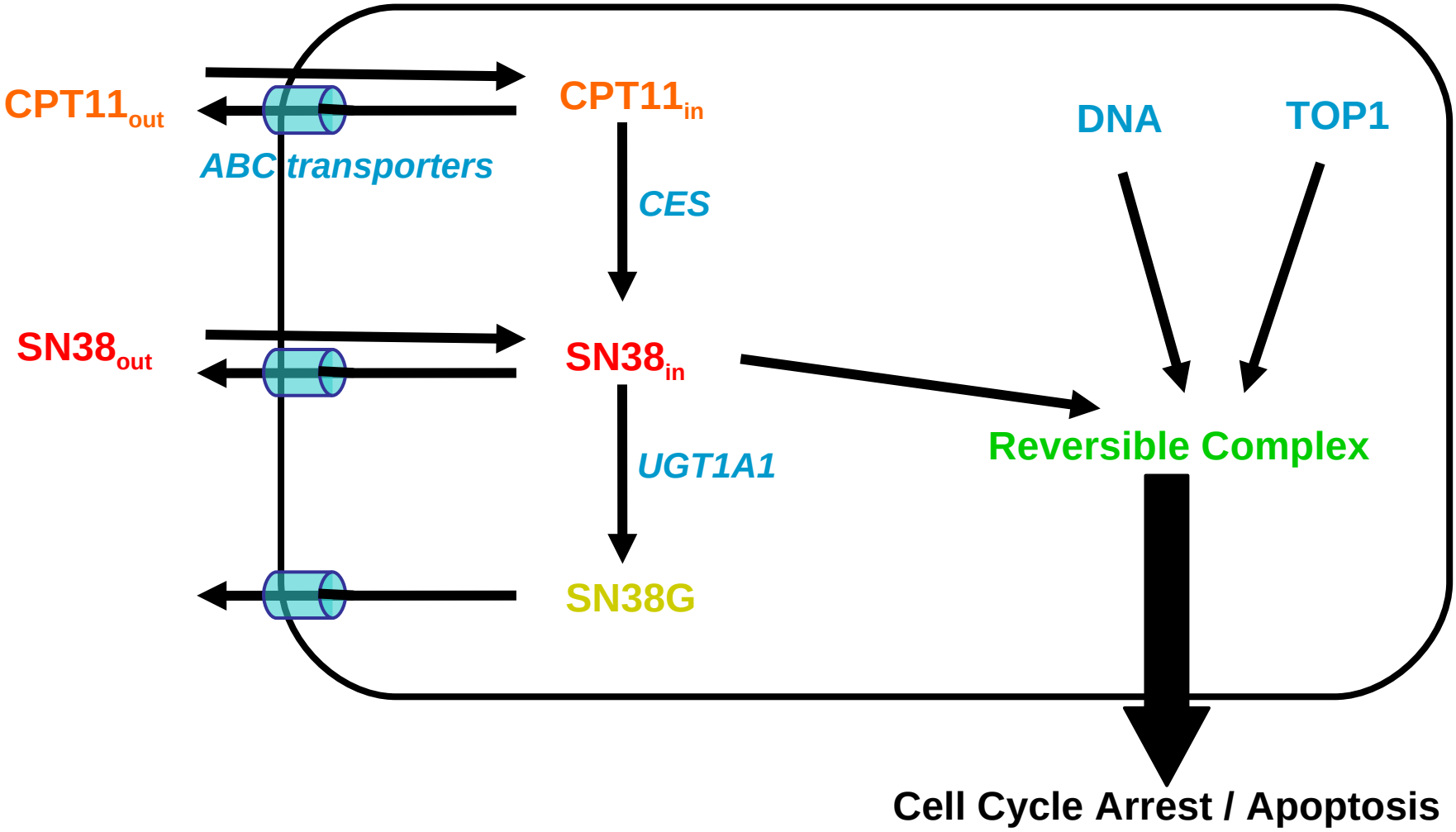


- Others have found circadian rhythm for Topoisomerase I (cf. *Circadian regulation of mouse topoisomerase I gene expression by glucocorticoid hormones,* Y. Kuramoto and al., Biochemical Pharmacology, 2006)

Mathematical Modeling



Mathematical Modeling



Mathematical Modeling

System of Equations:

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

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

$$\frac{d[SN38_{out}]}{dt} = -k_{uptakeSN}[SN38_{out}] + \frac{V_{effSN}[ABC][SN38_{in}]}{K_{effSN}\frac{V_{out}}{V_{in}} + V_{out}[SN38_{in}]}$$

$$\frac{d[SN38_{in}]}{dt} = k_{uptakeSN}\frac{V_{out}}{V_{in}}[SN38_{out}] - \frac{V_{effSN}[ABC][SN38_{in}]}{K_{effSN} + V_{in}[SN38_{in}]} + \frac{V_{CPT-SN}[CPT11_{in}]}{K_{CPT-SN} + [CPT11_{in}]}$$
$$- \frac{V_{SN-SNG}[UGT][SN38_{in}]}{K_{SN-SNG} + [SN38_{in}]} - kf_C[TOP1][SN38_{in}](DNA_{tot} - [COMPL]) + kr_C[COMPL]$$

$$\frac{d[SN38G]}{dt} = \frac{V_{SN-SNG}[UGT][SN38_{in}]}{K_{SN-SNG} + [SN38_{in}]} - \frac{V_{effSNG}[ABC][SN38G]}{K_{effSNG} + V_{in}[SN38G]}$$

$$\frac{d[COMPL]}{dt} = kf_C[TOP1][SN38_{in}](DNA_{tot} - [COMPL]) - kr_C[COMPL]$$

Resistance

$$\frac{d[NF]}{dt} = \frac{[SN38_{in}]^n}{K_{ind}^n + [SN38_{in}]^n} - k_{dNF}[NF]$$

$$\frac{d[ABC]}{dt} = k_{fABC} + k_{ind} * [NF] - k_{dABC}[ABC]$$

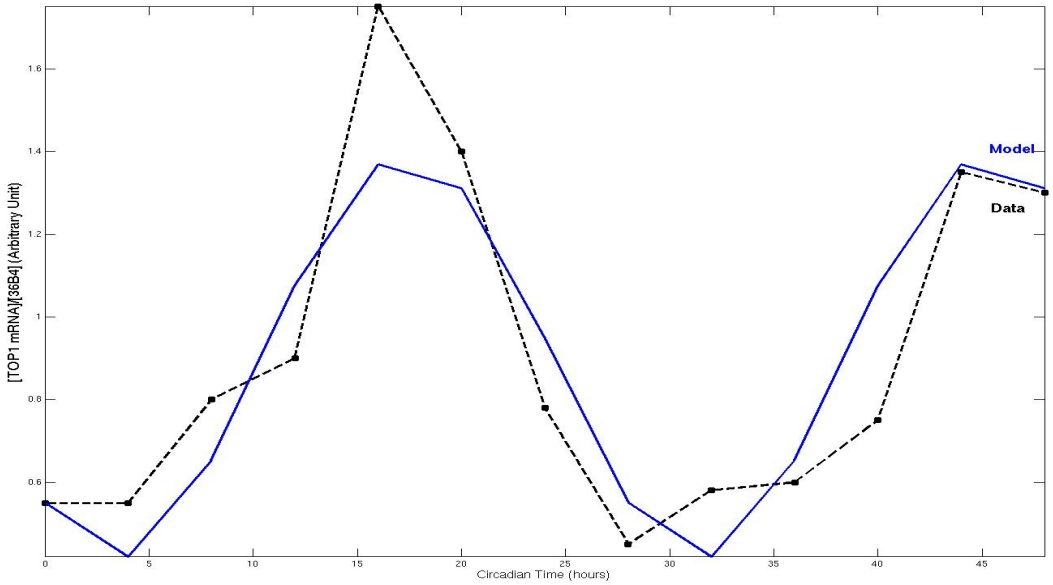
Circadian Rhythm

$$\frac{d[TOP1]}{dt} = k_{fTOP}(1 + \epsilon_{TOP}cos(\frac{2\pi}{24}(t - \phi_{TOP}))) - k_{dTOP}[TOP1]$$
$$-kf_C[TOP1][SN38_{in}](DNA_{tot} - [COMPL]) + kr_C[COMPL]$$

$$\frac{d[UGT1A1]}{dt} = k_{fUGT}(1 + \epsilon_{UGT}cos(\frac{2\pi}{24}(t - \phi_{UGT}))) - k_{dUGT}[UGT]$$

Mathematical Modeling

Parameters for TOPI and UGT1A1 have been chosen to fit the data obtained in Caco-2 cells:

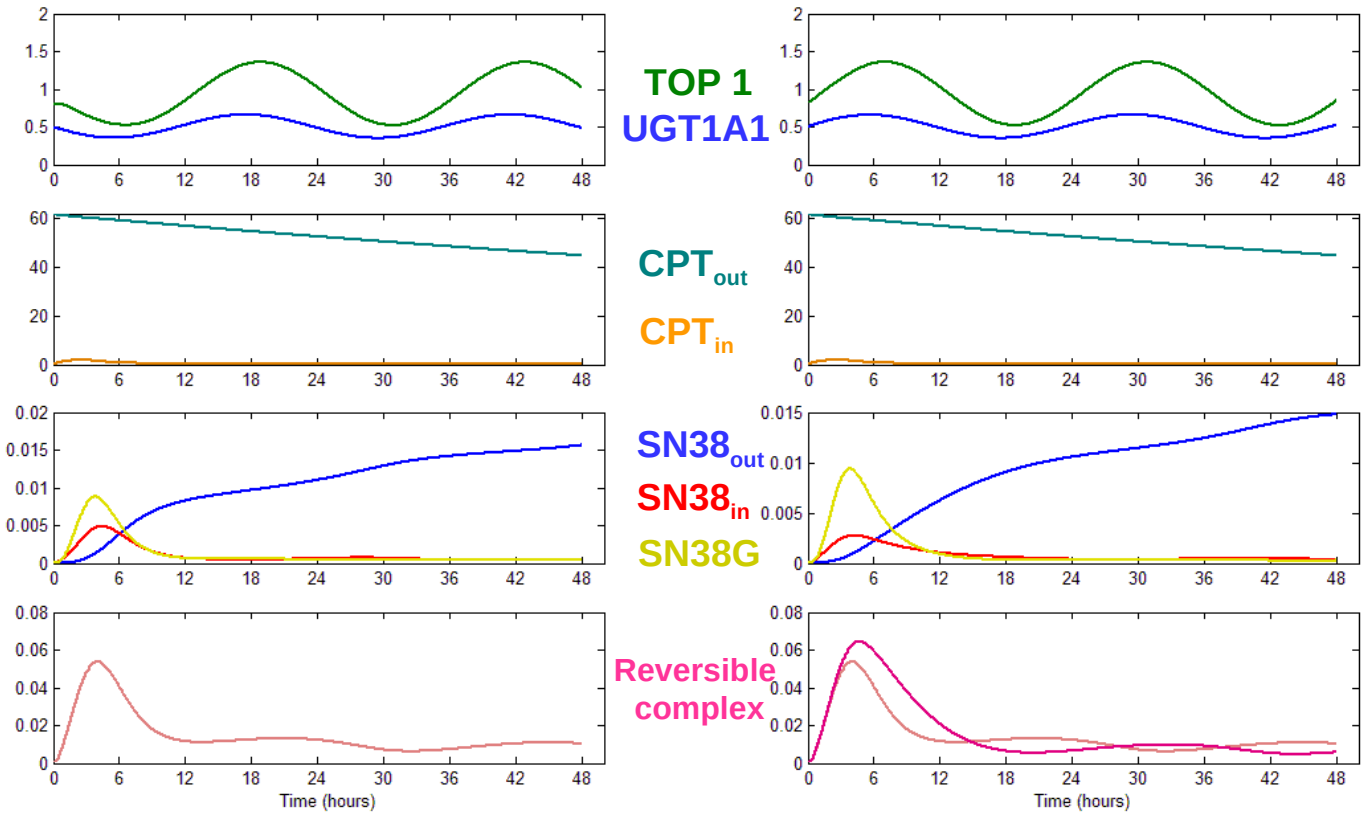


Mathematical Modeling

Simulation: chosing the right circadian time to expose cells

Exposition in phase with TOP1

Exposition out of phase with TOP1



Conclusion and future work

- The decrease in CPT11 intracellular accumulation over time may be explained by the induction of ABC transporters. Further work is in progress to validate this hypothesis.

- Circadian rhythms of ABC transporters are being studied.
- Data about SN38 glucuronidation and about formation of reversible complexes are needed.

- This study at the cell population scale may then be integrated into a Whole-Body approach leading to potential improvements in the administration of Irinotecan to patients.