Characterization of the active efflux of fluoroquinolones in eukaryotic cells

Coralie VALLET

Promotor: Françoise Van Bambeke

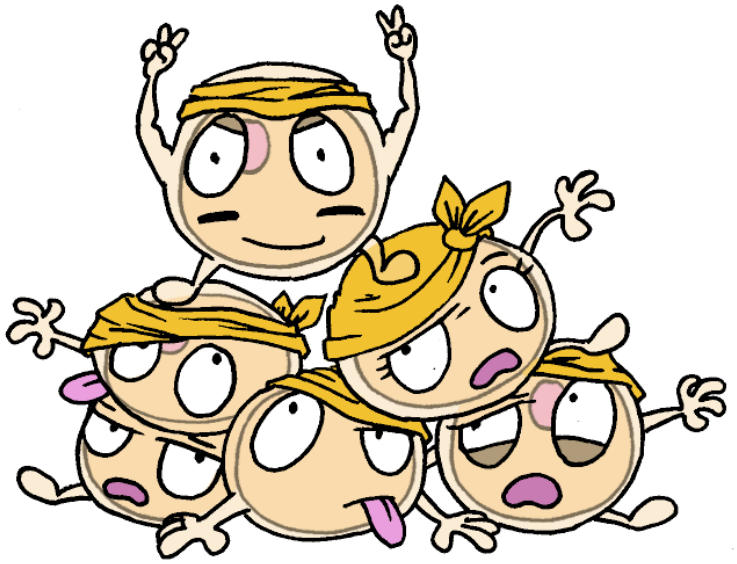
INTRODUCTION

RESISTANCE: What? When? Why?

- Defence mechanism against cellular invasion by toxic substances.

- Chronic use of chemotherapy leads to resistance
  - anticancer agents ⟶ eukaryotic cells
  - antibiotics ⟶ prokaryotic cells (bacteria)

- Cell adaptation in order to avoid death



INTRODUCTION

RESISTANCE: How?

- enzymes ⇨ inactive drugs
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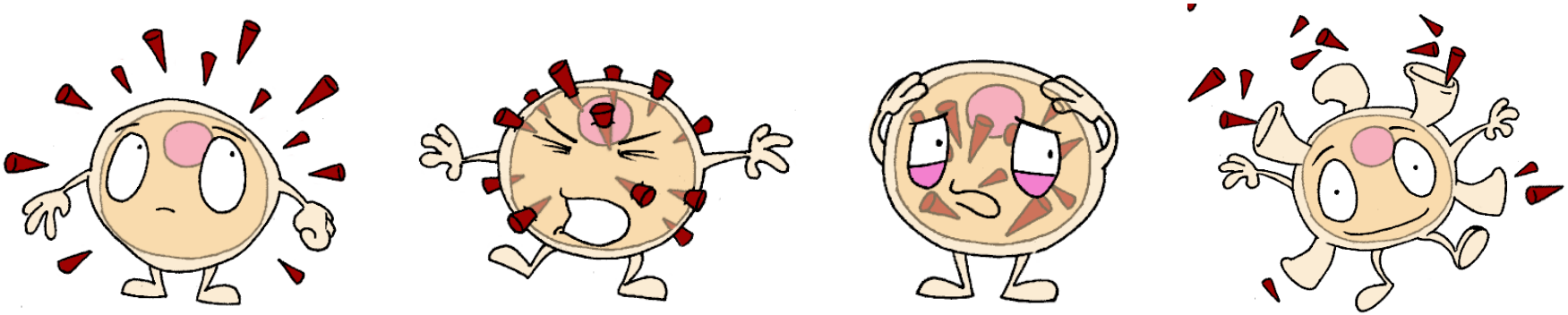

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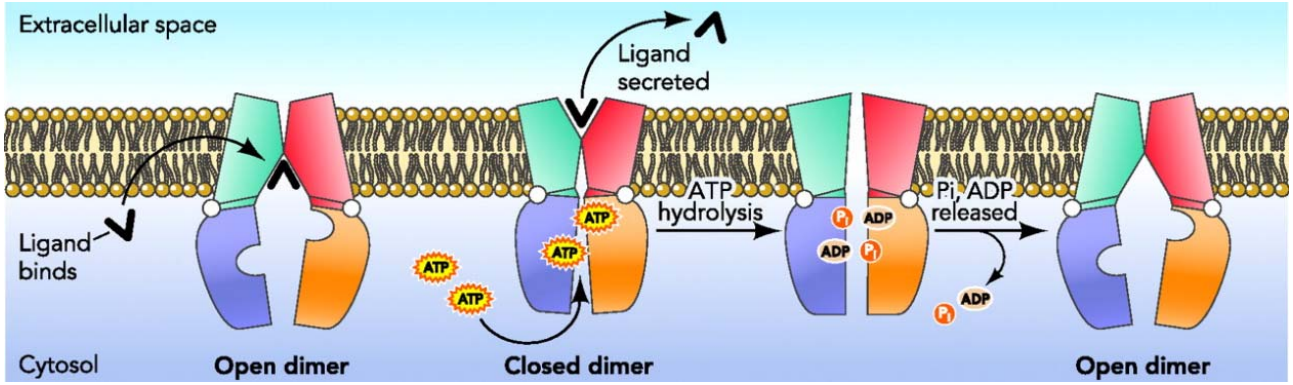
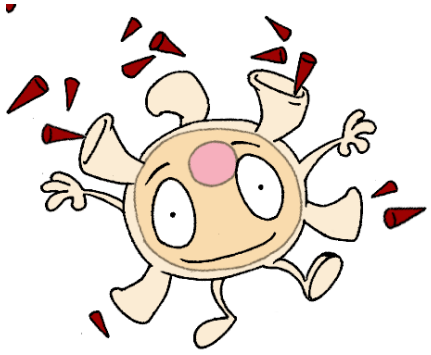
  ABC (ATP Binding Cassette) transporters





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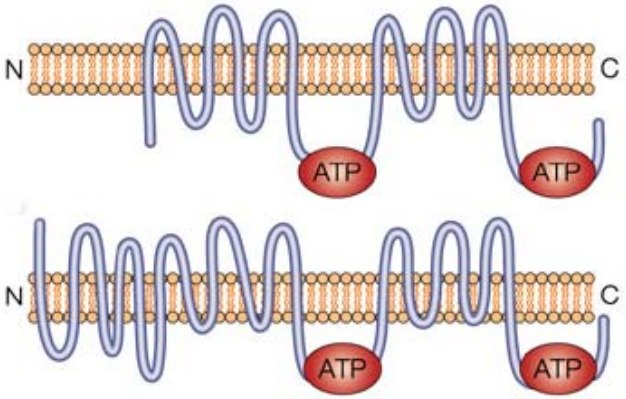
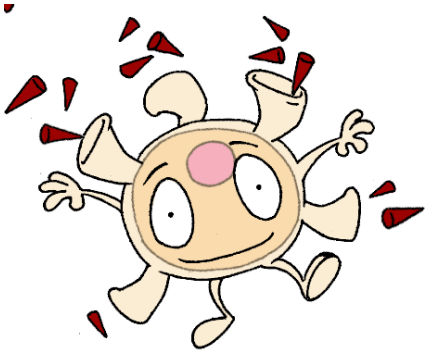
  ABC (ATP Binding Cassette) transporters
  MRP = Multidrug resistance associated proteins



MDR1 (ABCB1)
MRP4 (ABCC4)
MRP5 (ABCC5)
MRP7 (ABCC1)

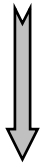
MRP1 (ABCC1)
MRP2 (ABCC2)
MRP3 (ABCC3)
MRP6 (ABCC6)



INTRODUCTION

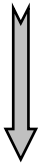
Why study the active efflux of **antibiotics** in **eukaryotic** cells?

antibiotics



Prokaryotic cells
(bacteria)

Anticancer agents



Eukaryotic cells
(animal)

INTRODUCTION

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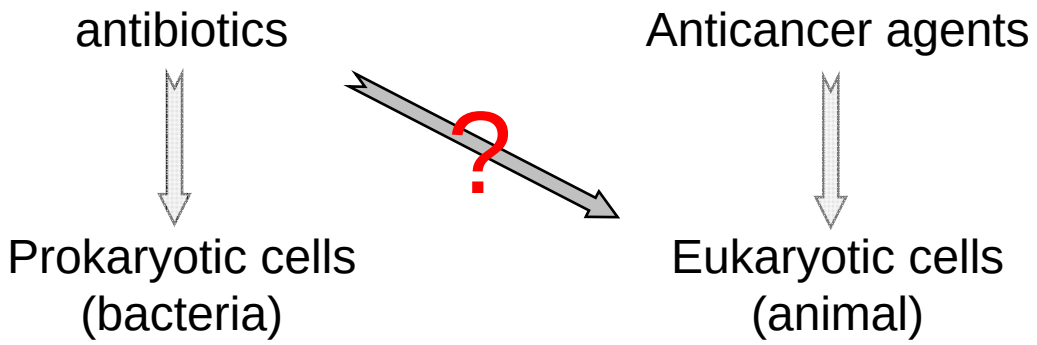

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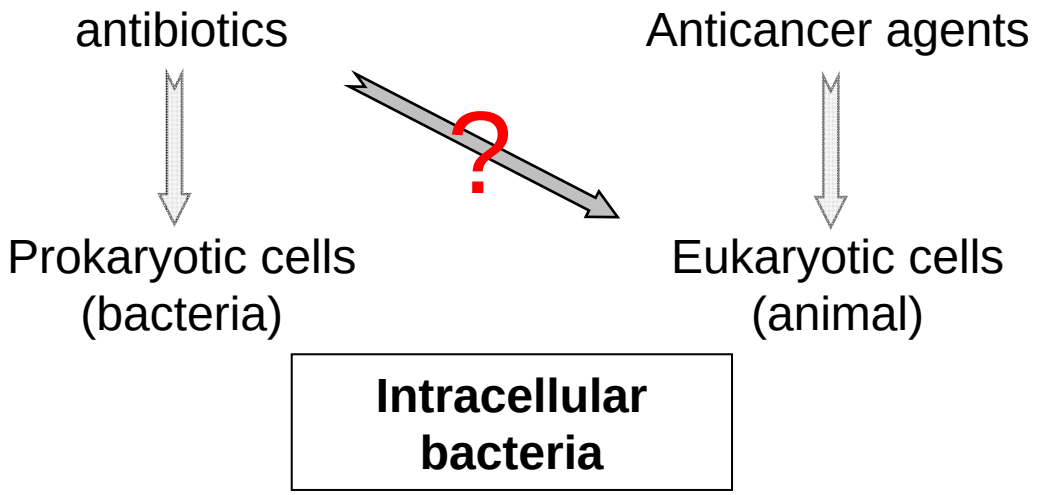
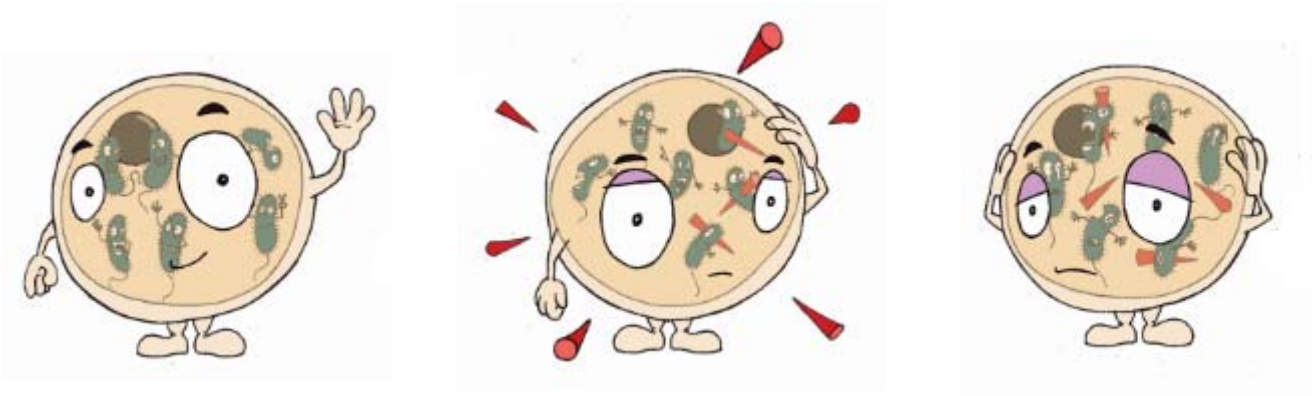
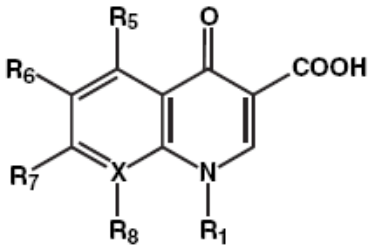
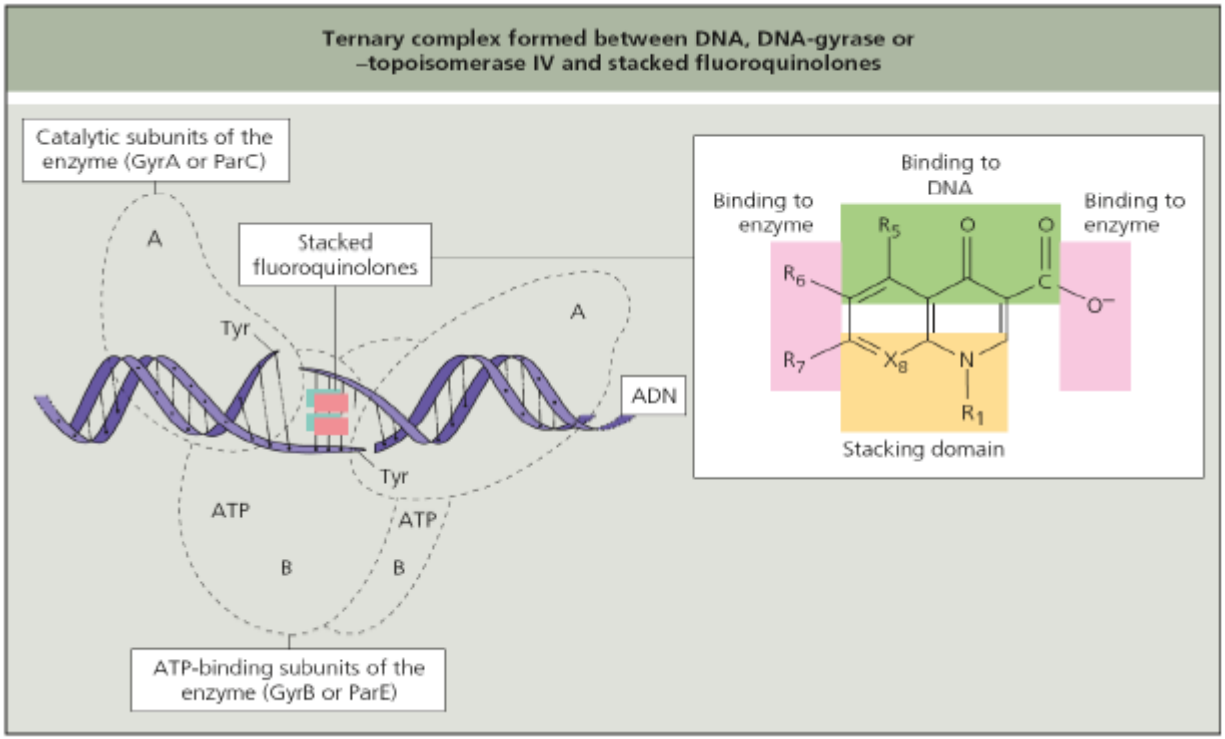




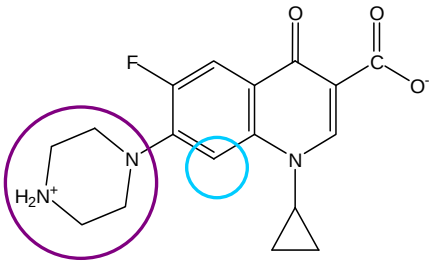
Antibiotics = fluoroquinolones



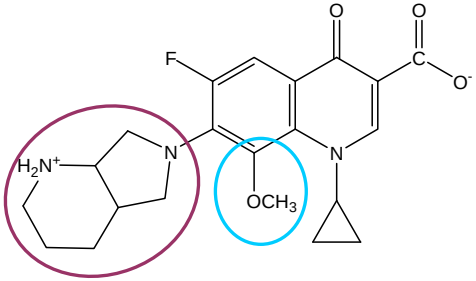
Inhibition of topoisomerase activity ⇨ bacteria's death



Characterization of fluoroquinolone efflux in macrophages



CIPROFLOXACIN



MOXIFLOXACIN

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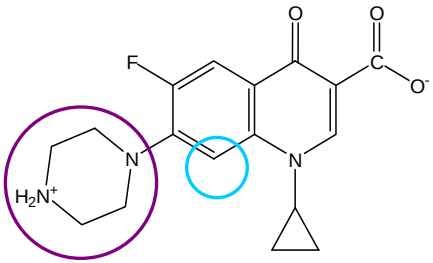

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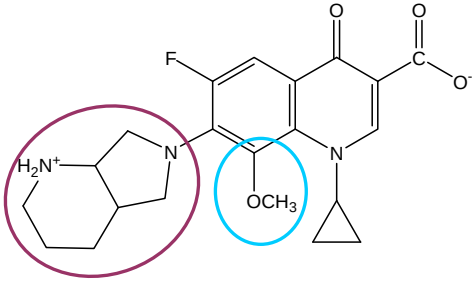

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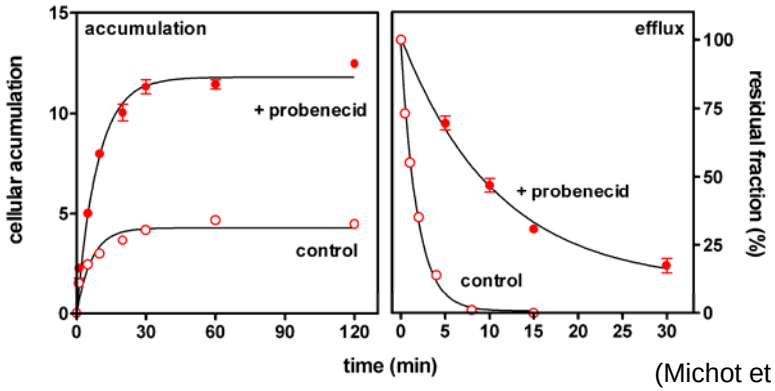
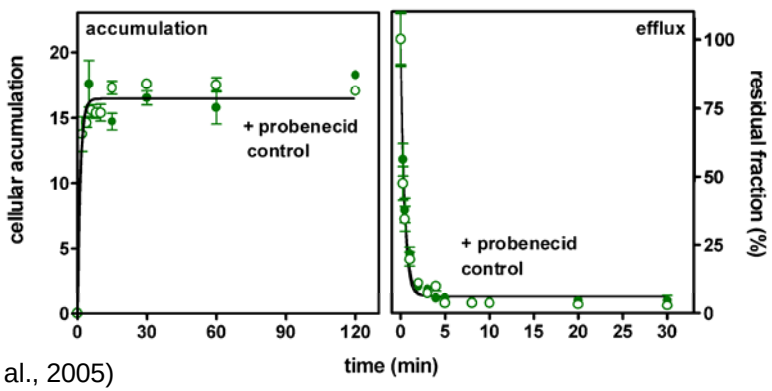
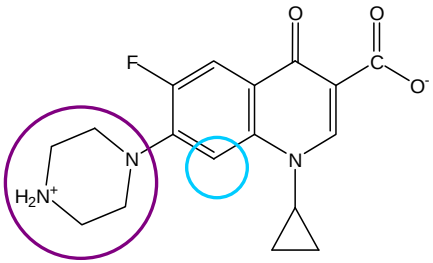




(Michot et al., 2004 ; Michot et al., 2005)

Characterization of fluoroquinolone efflux in macrophages



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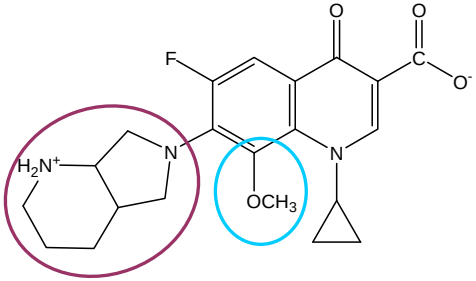

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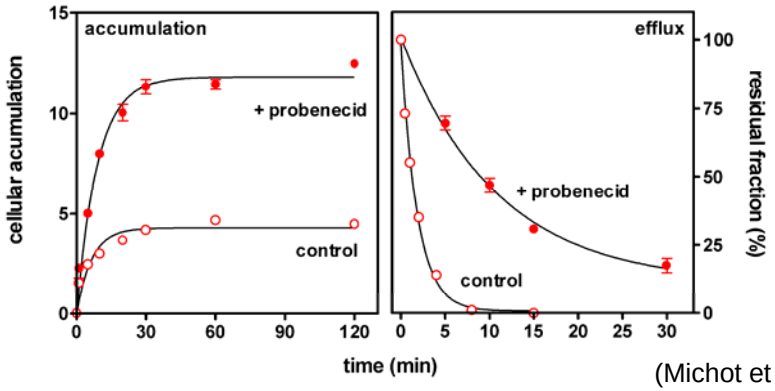
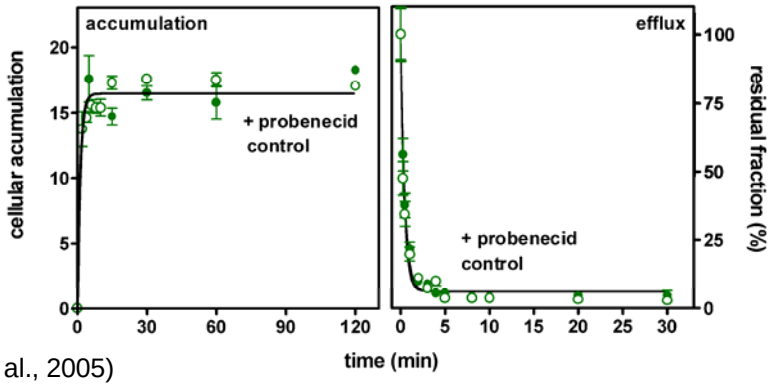




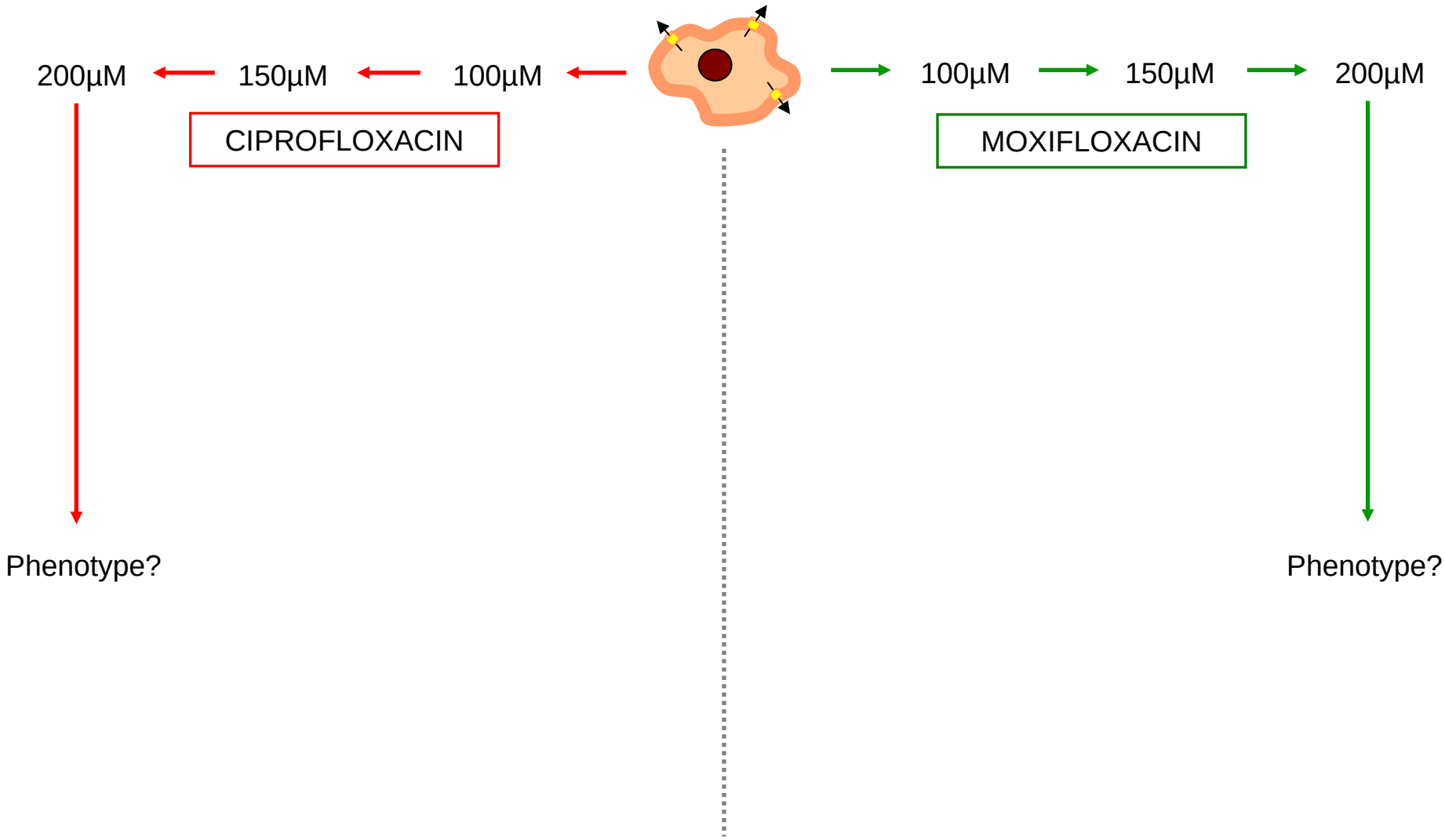
(Michot et al., 2004 ; Michot et al., 2005)

MRP substrate

Non MRP substrate

CONTEXT OF THE STUDY

Can we make eukaryotic cells resistant to fluoroquinolones?



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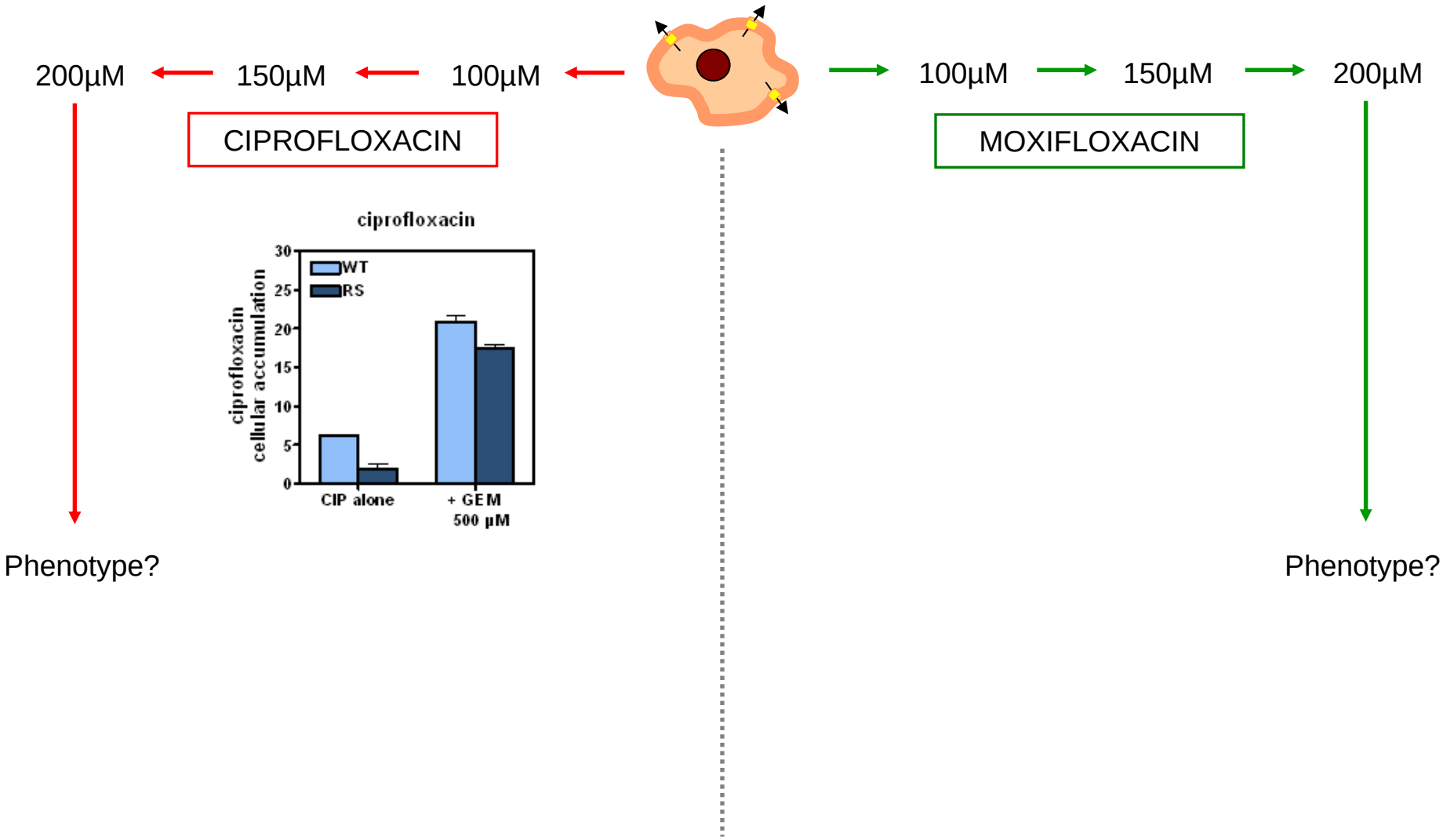


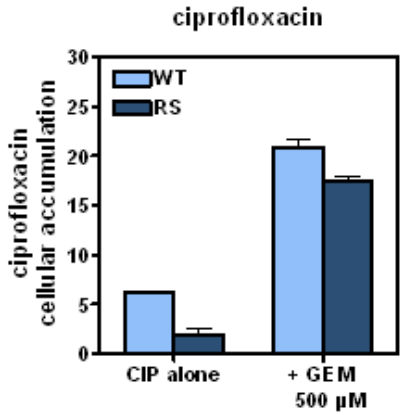
CONTEXT OF THE STUDY

Can we make eukaryotic cells resistant to fluoroquinolones?

200µM ← 150µM ← 100µM ← → 100µM → 150µM → 200µM

CIPROFLOXACIN

MOXIFLOXACIN

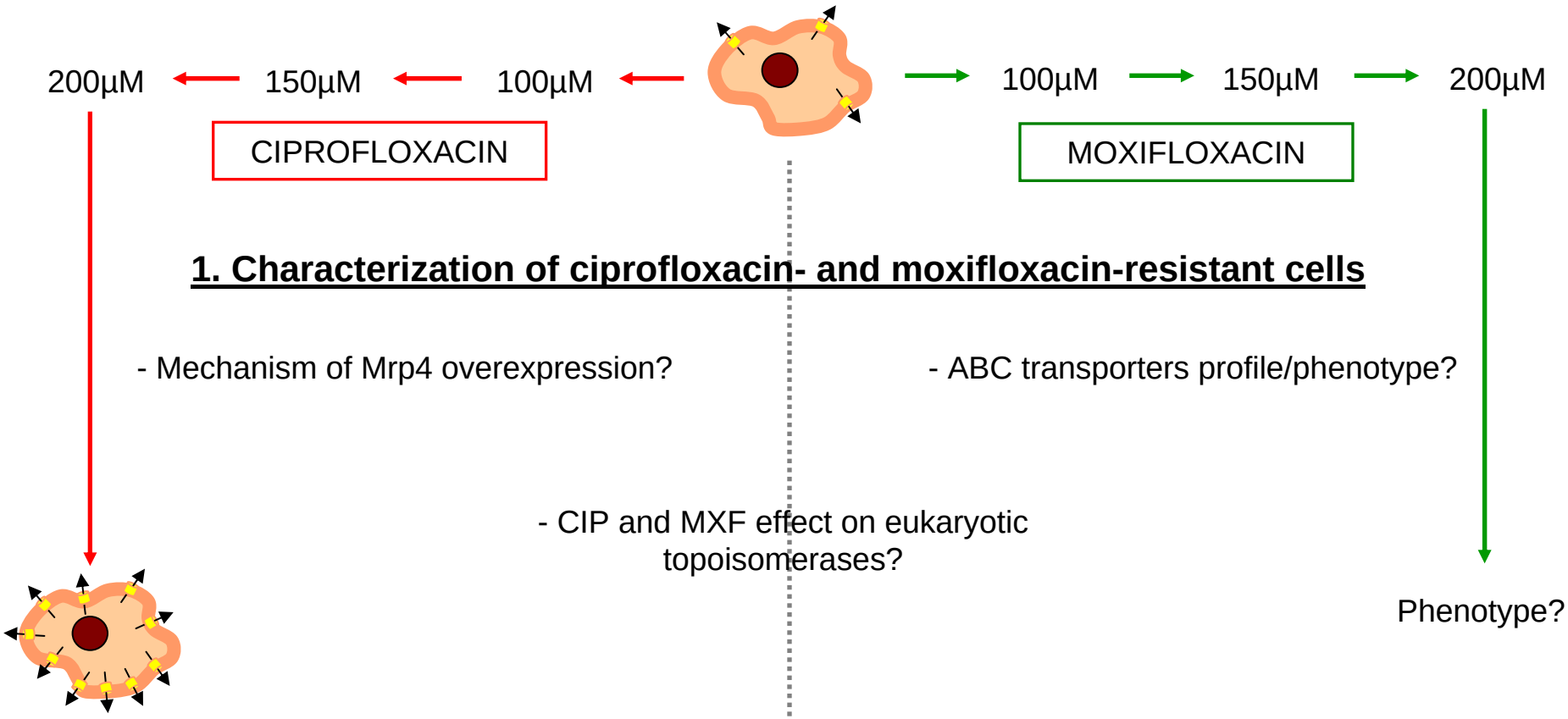


Phenotype?

Mrp4 overexpression

☞ CIP accumulation ↘

OBJECTIVES OF THE STUDY



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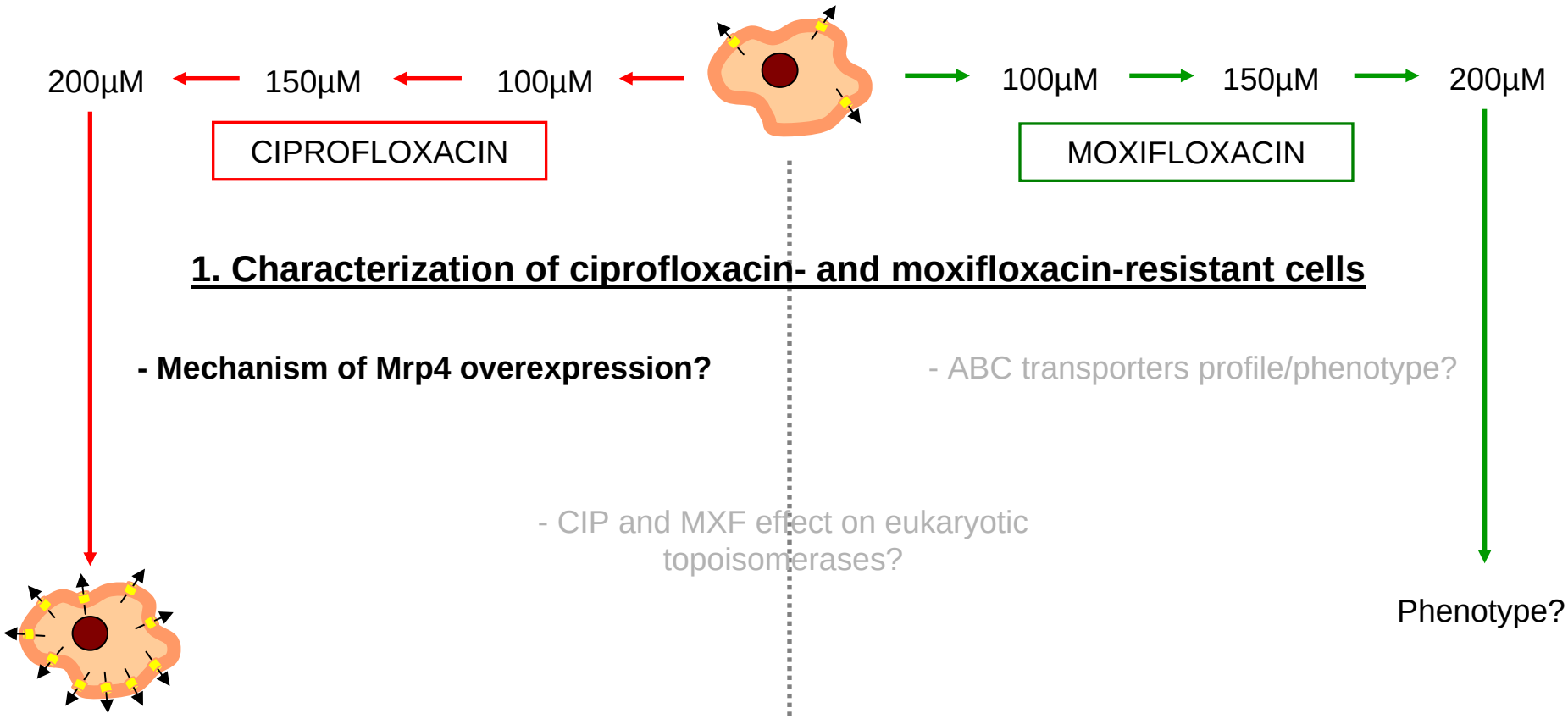

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PLoS one

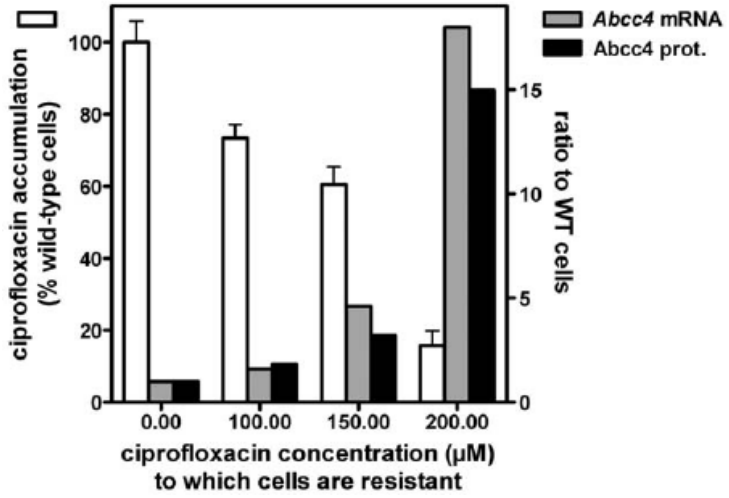
Characterization of *Abcc4* Gene Amplification in Stepwise-Selected Mouse J774 Macrophages Resistant to the Topoisomerase II Inhibitor Ciprofloxacin

Béatrice Marquez[1¤], Geneviève Ameye[2], Coralie M. Vallet[1], Paul M. Tulkens[1], Hélène A. Poirel[2], Françoise Van Bambeke[1*]

1 Université catholique de Louvain, Louvain Drug Research Institute, Pharmacologie cellulaire et moléculaire, Brussels, Belgium, **2** Université catholique de Louvain, Cliniques universitaires Saint-Luc, Centre de Génétique humaine, Brussels, Belgium

RESULTS I: resistance and mechanisms

CIP accumulation and Mrp4 expression



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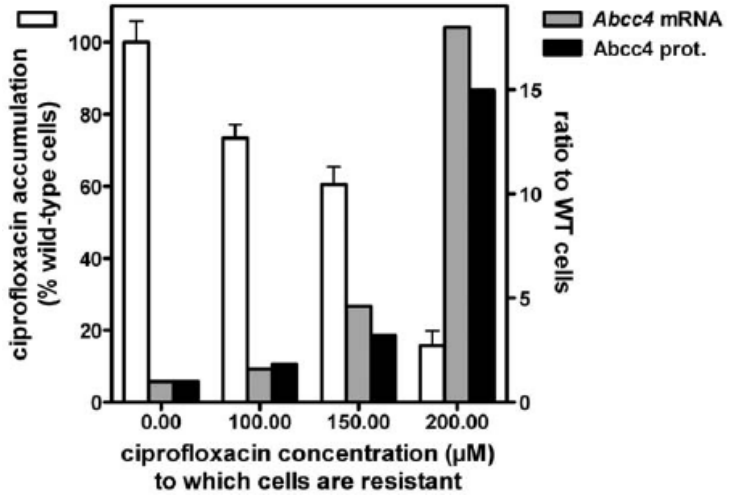

↘ CIP accumulation

↗ Mrp4 (gene and protein)

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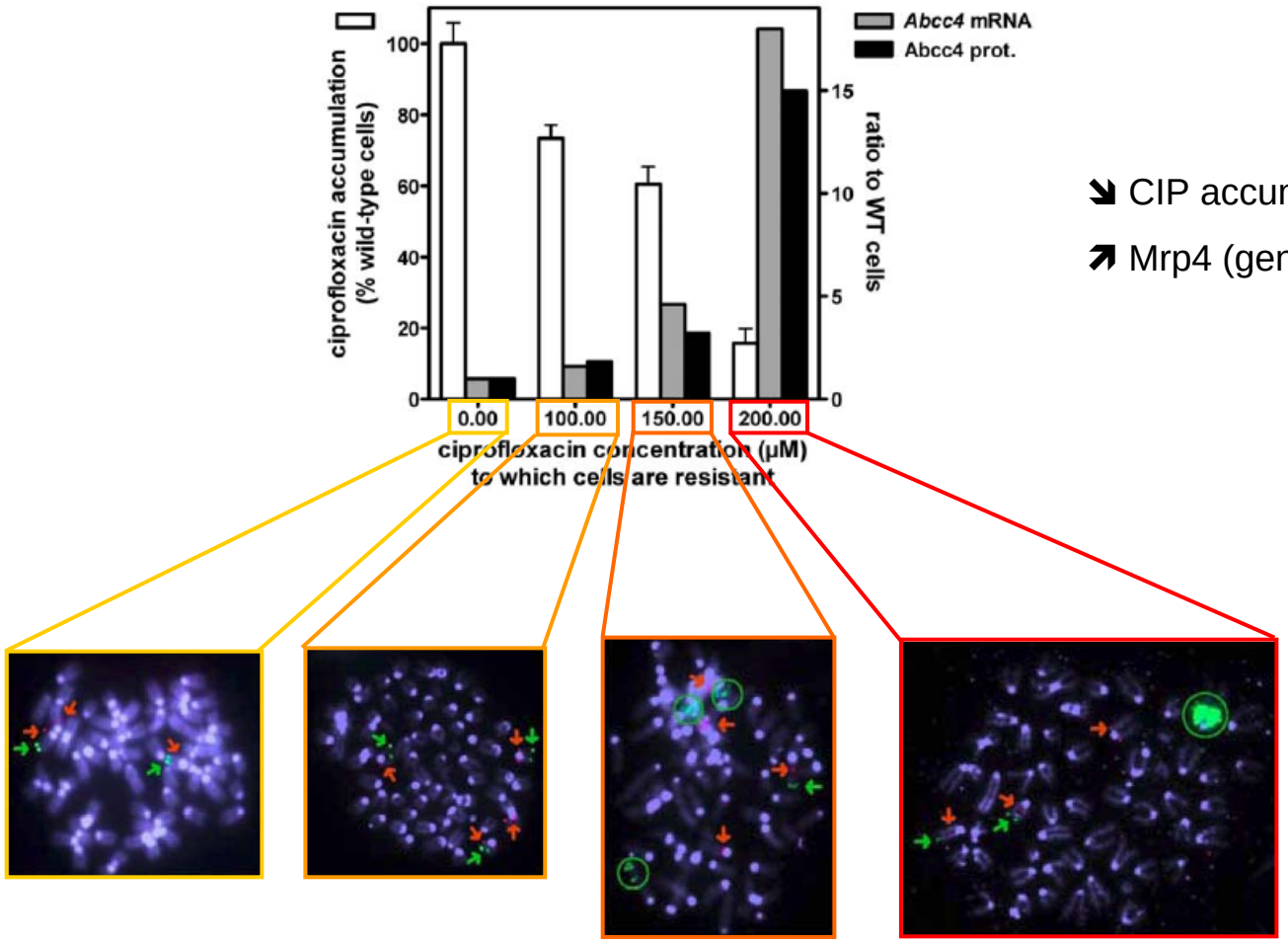
CIP accumulation and Mrp4 expression



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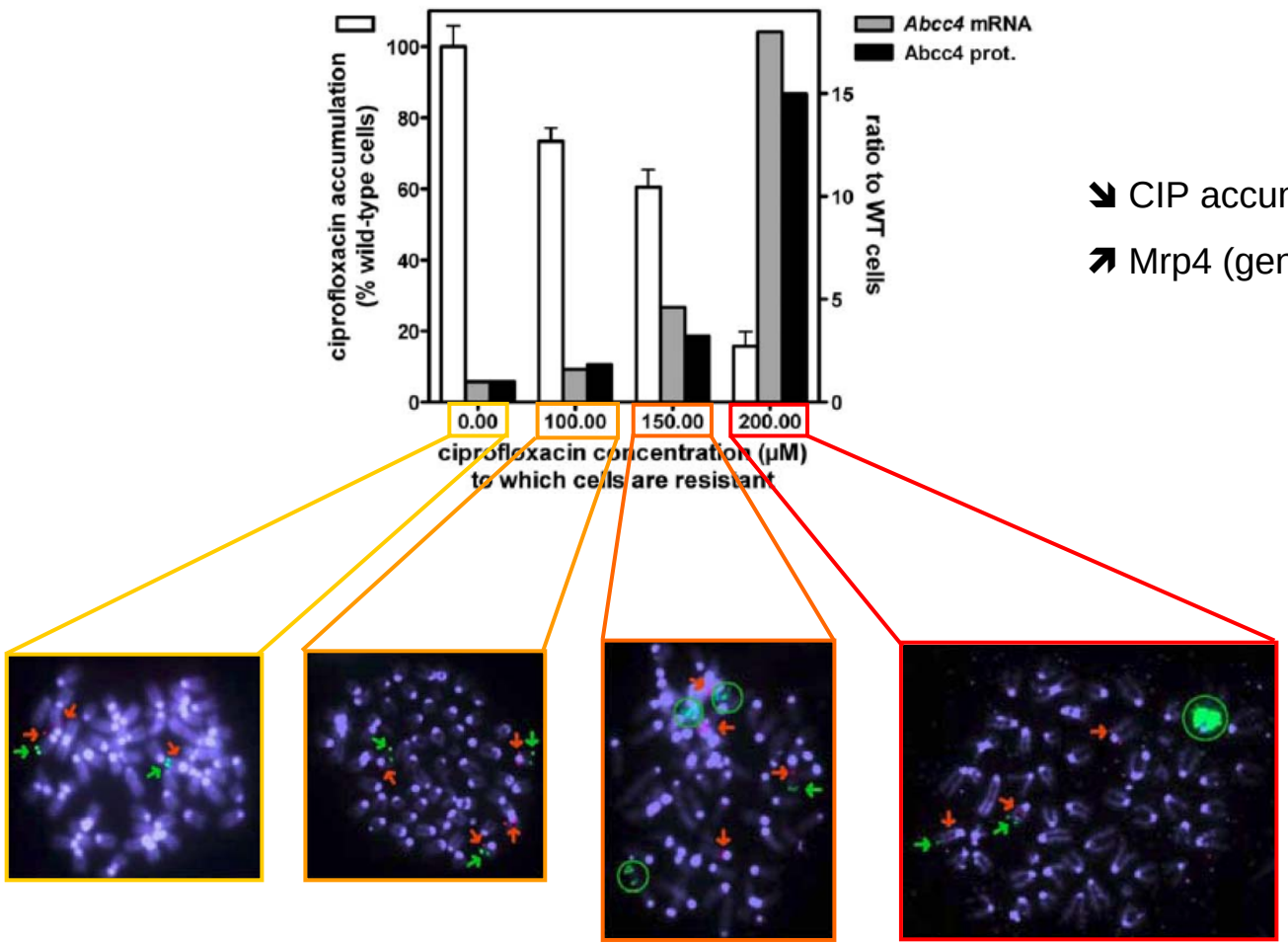
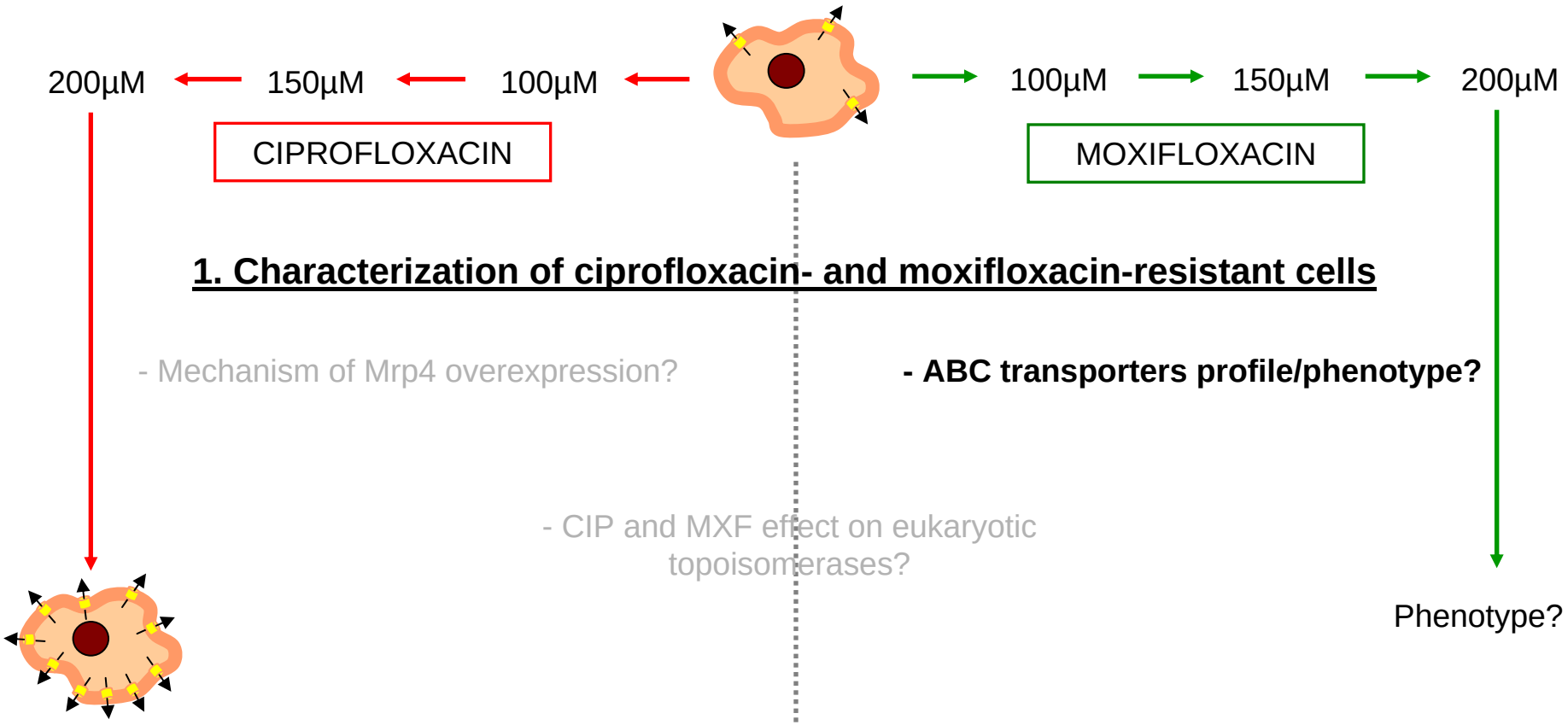


↘ CIP accumulation

↗ Mrp4 (gene and protein)

Over-representation of the Mrp4 protein in CIP-R cells
⇨ due to a duplication of the *mrp4* gene

SUMMARY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RESULTS I: resistance and mechanisms

Toxicology 290 (2011) 178–186

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

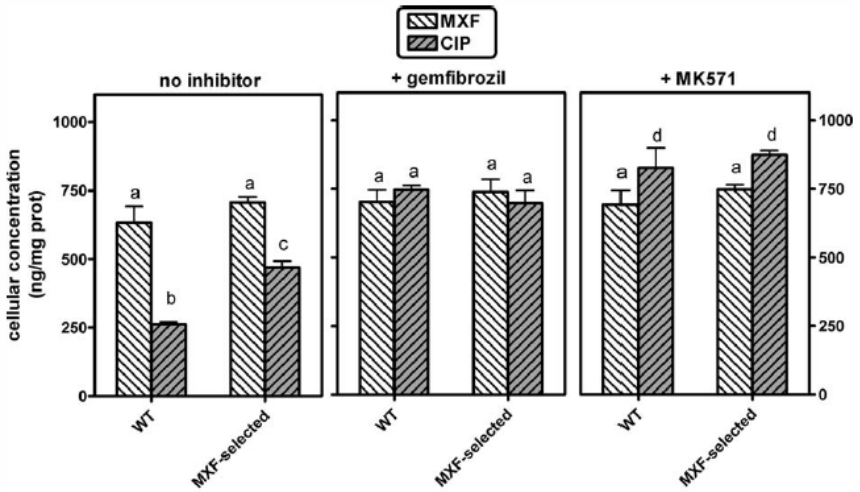
Modulation of the expression of ABC transporters in murine (J774) macrophages exposed to large concentrations of the fluoroquinolone antibiotic moxifloxacin

Coralie M. Vallet[a,1], Béatrice Marquez[a,1,2], Naïma Nhiri[b], Ahalieyah Anantharajah[a], Marie-Paule Mingeot-Leclercq[a], Paul M. Tulkens[a], Jean-Yves Lallemand[b], Eric Jacquet[b,c], Françoise Van Bambeke[a,*]

[a] *Université catholique de Louvain, Louvain Drug Research Institute, Pharmacologie cellulaire et moléculaire, B-1200 Brussels, Belgium*
[b] *Centre de recherche de Gif, Institut de Chimie des Substances naturelles, avenue de la Terrasse, 91198 Gif-sur-Yvette, France*
[c] *IMAGIF qPCR Platform, CNRS UPR2301, avenue de la Terrasse, 91198 Gif-sur-Yvette, France*

RESULTS I: resistance and mechanisms

MXF and CIP accumulation

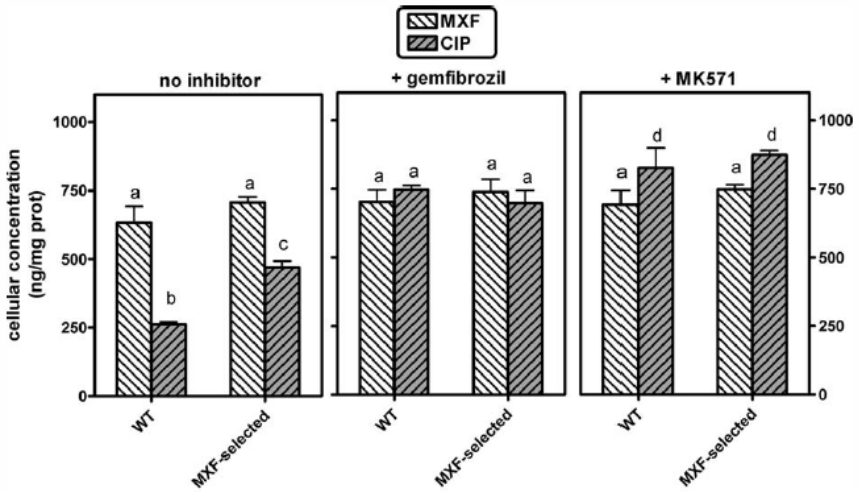


MXF accumulation constant

↗ CIP accumulation in MXF-R cells

RESULTS I: resistance and mechanisms

MXF and CIP accumulation



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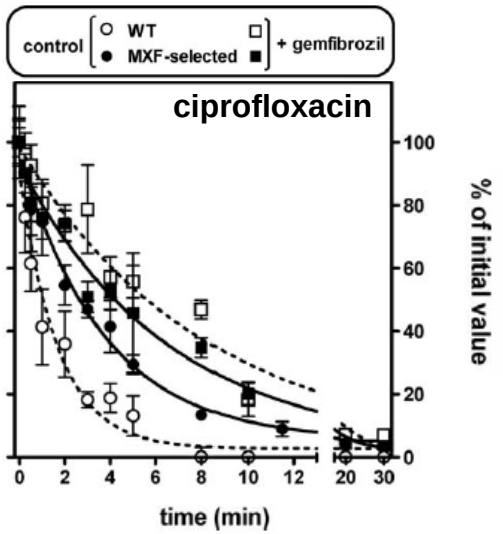
CIP efflux



CIP efflux slower in MXF-R cells

RESULTS I: resistance and mechanisms

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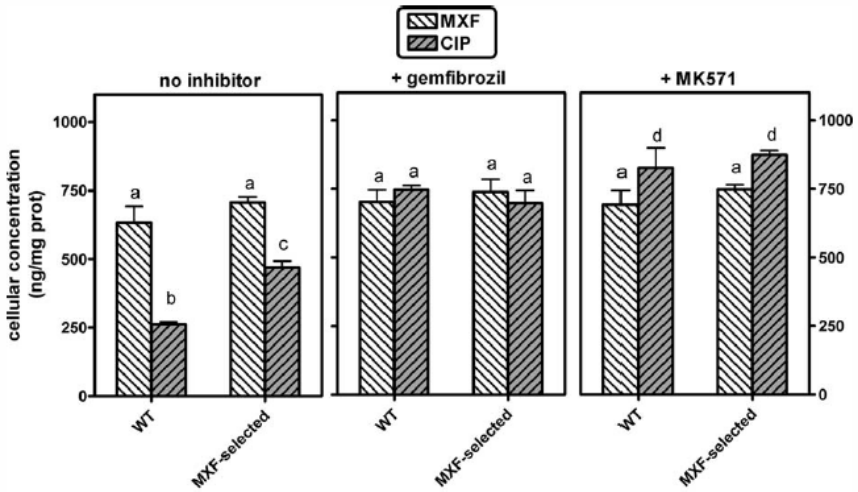

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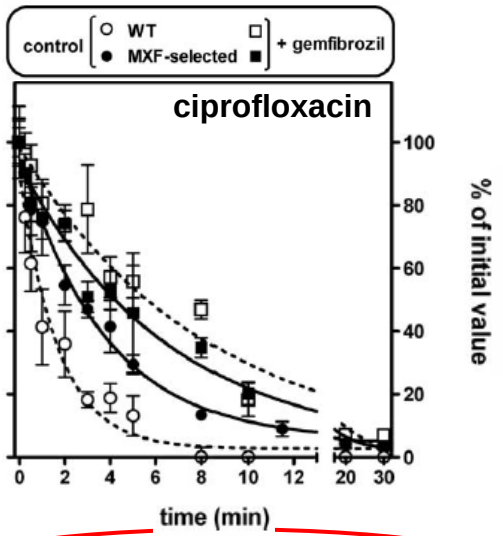

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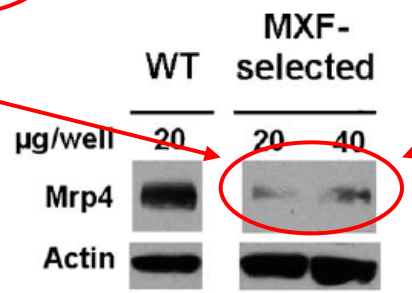
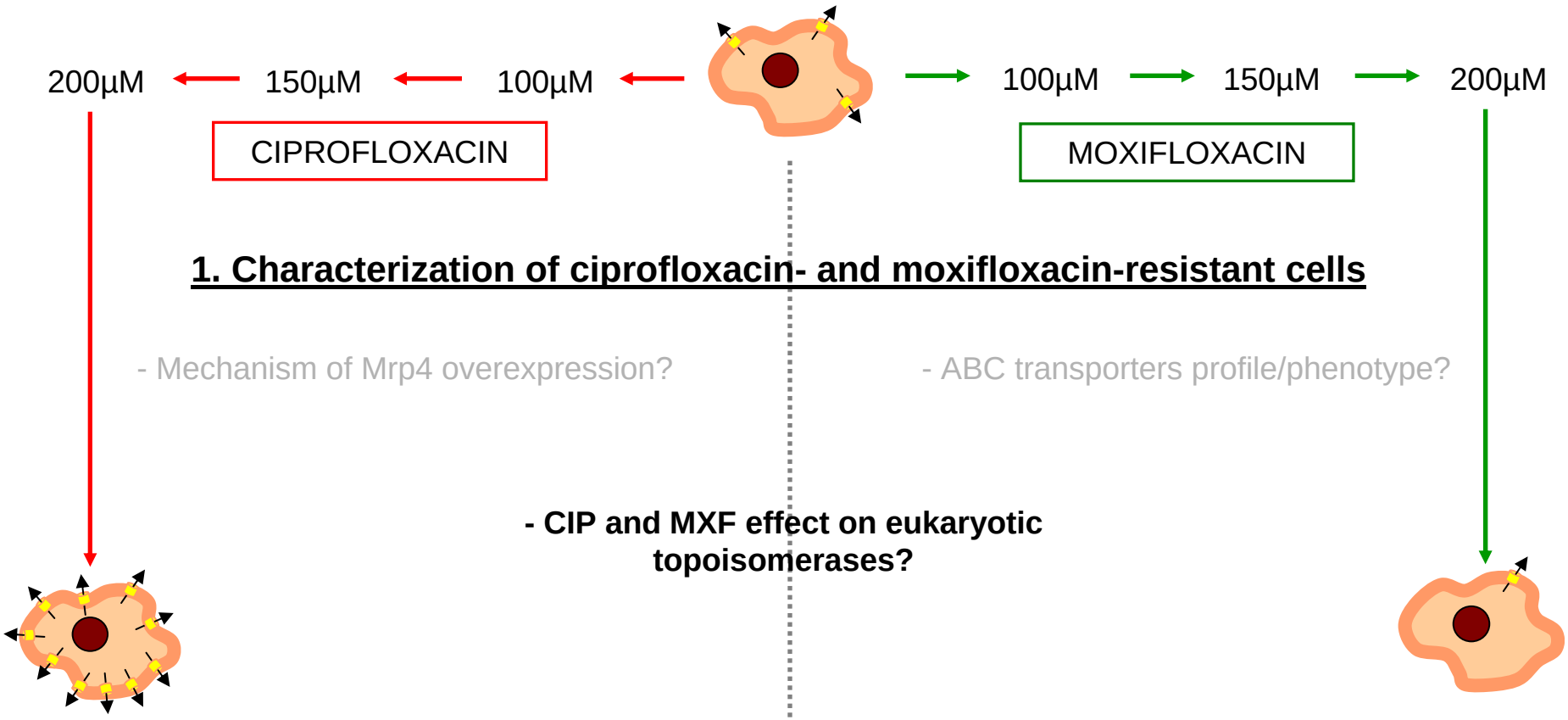


↘ Mrp4 protein in MXF-R cells

SUMMARY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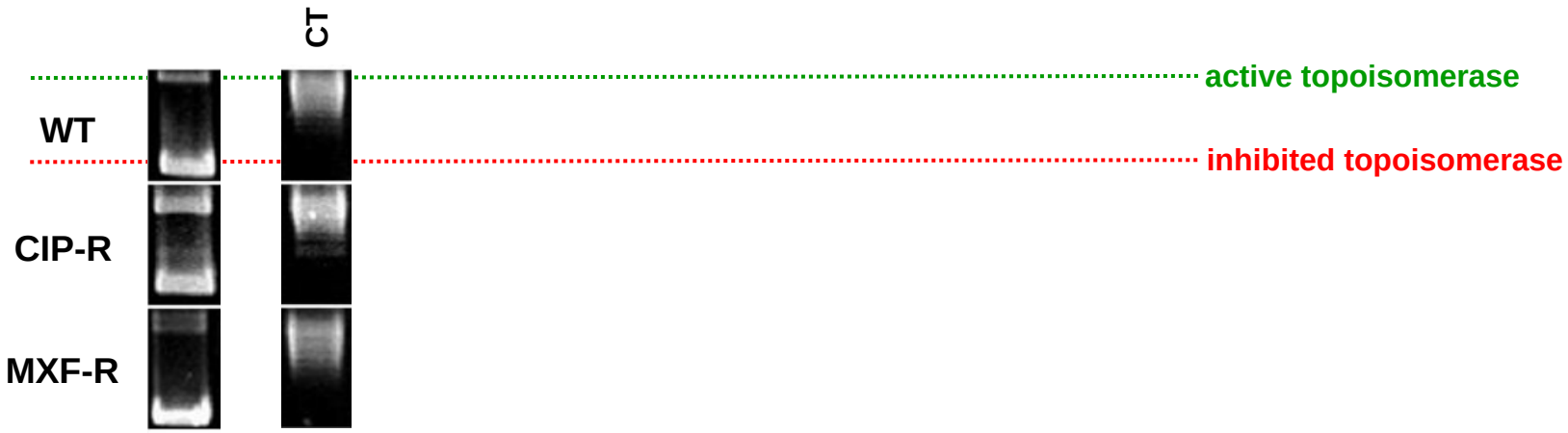
Topoisomerase activity in wild-type, ciprofloxacin- and moxifloxacin-resistant cells

RESULTS I: resistance and mechanisms

Topoisomerase activity in WT, CIP-R and MXF-R cells incubated with CIP, MXF, CPT, ETO or combination of anticancer agent and fluoroquinolones



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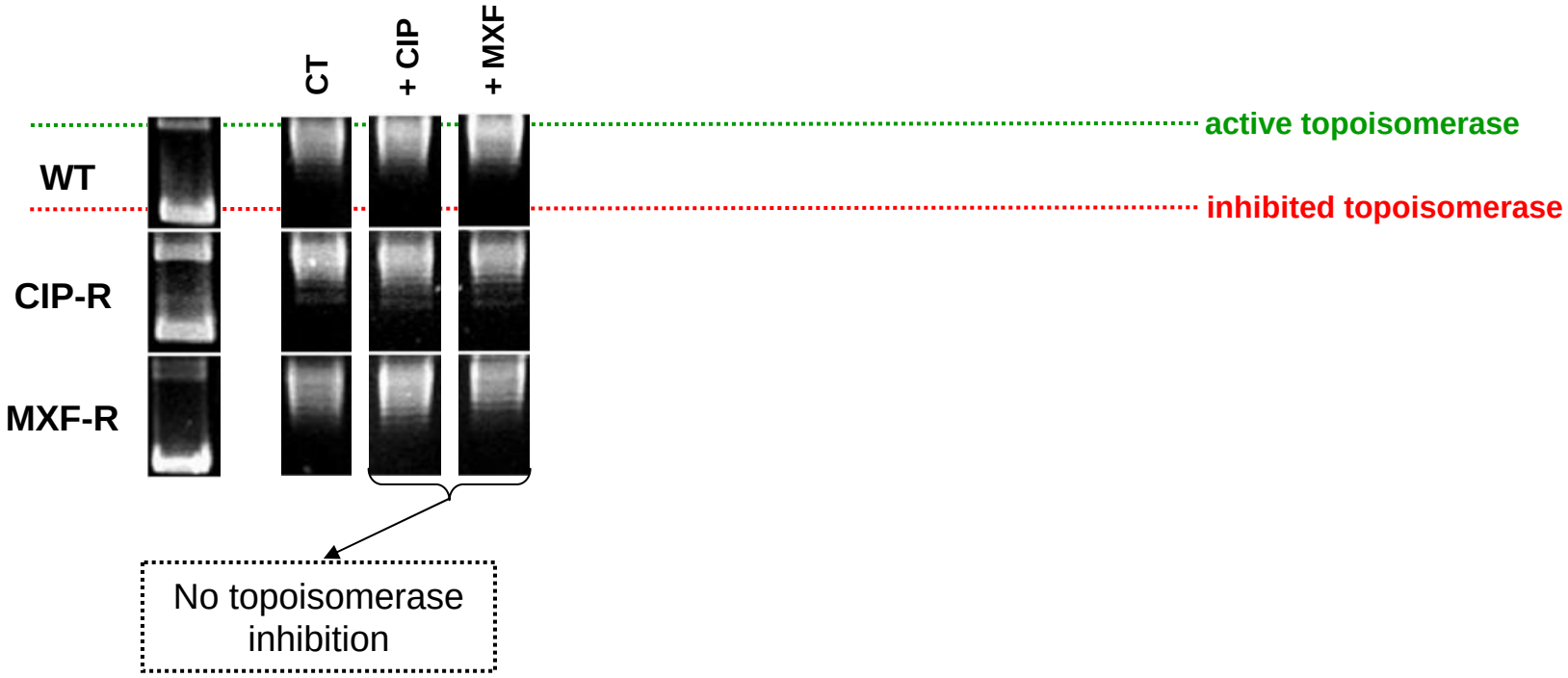

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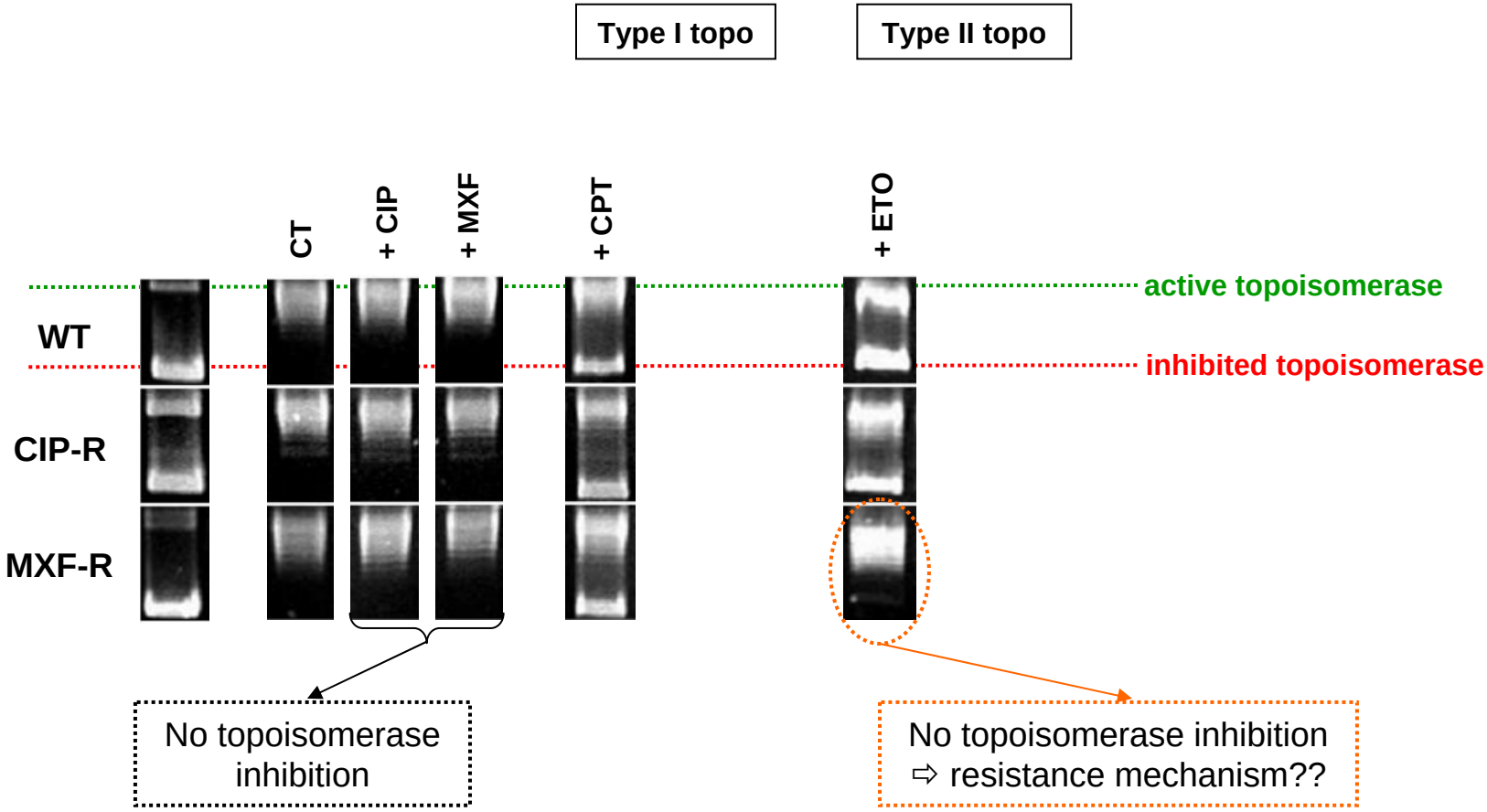

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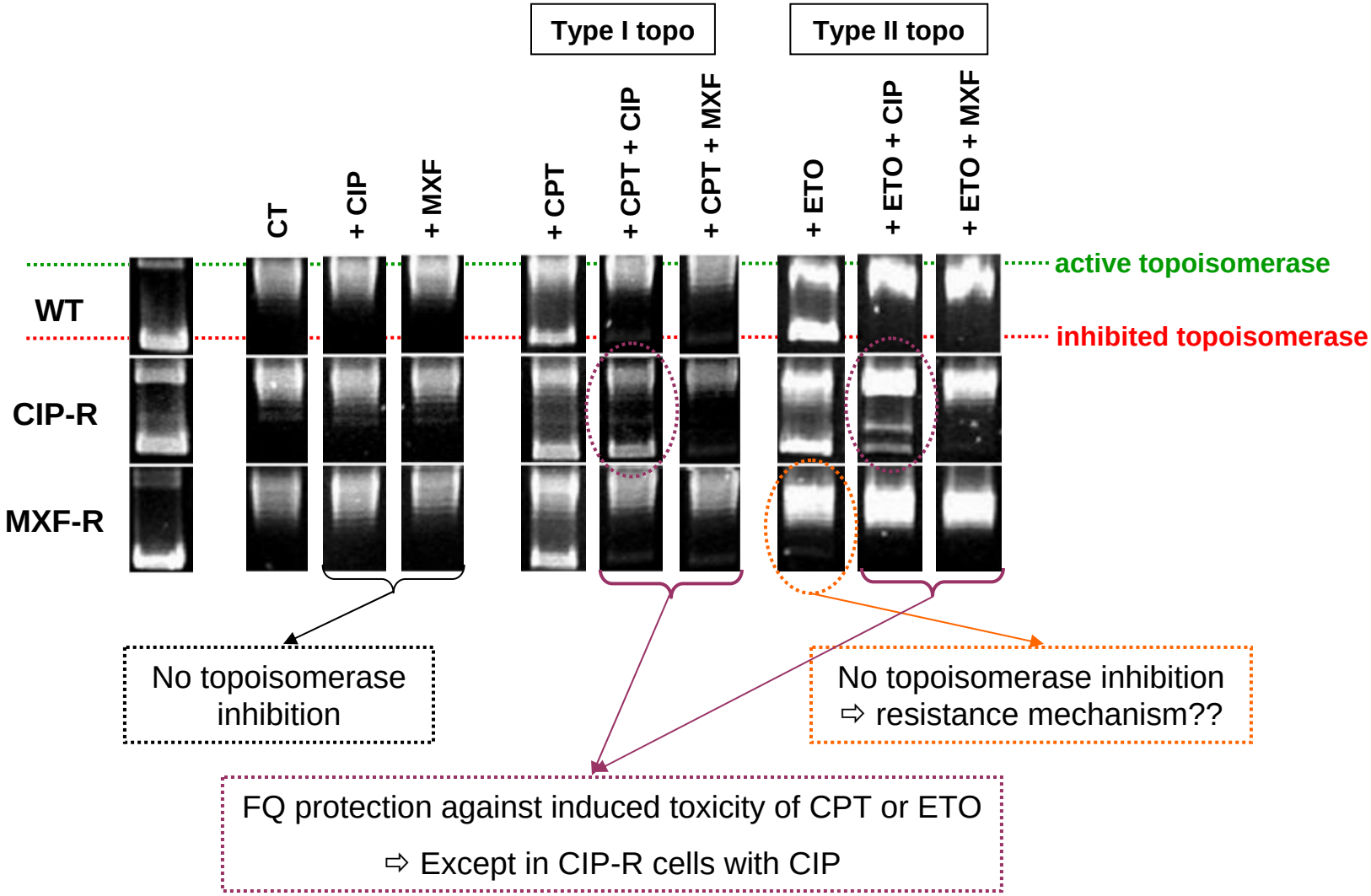

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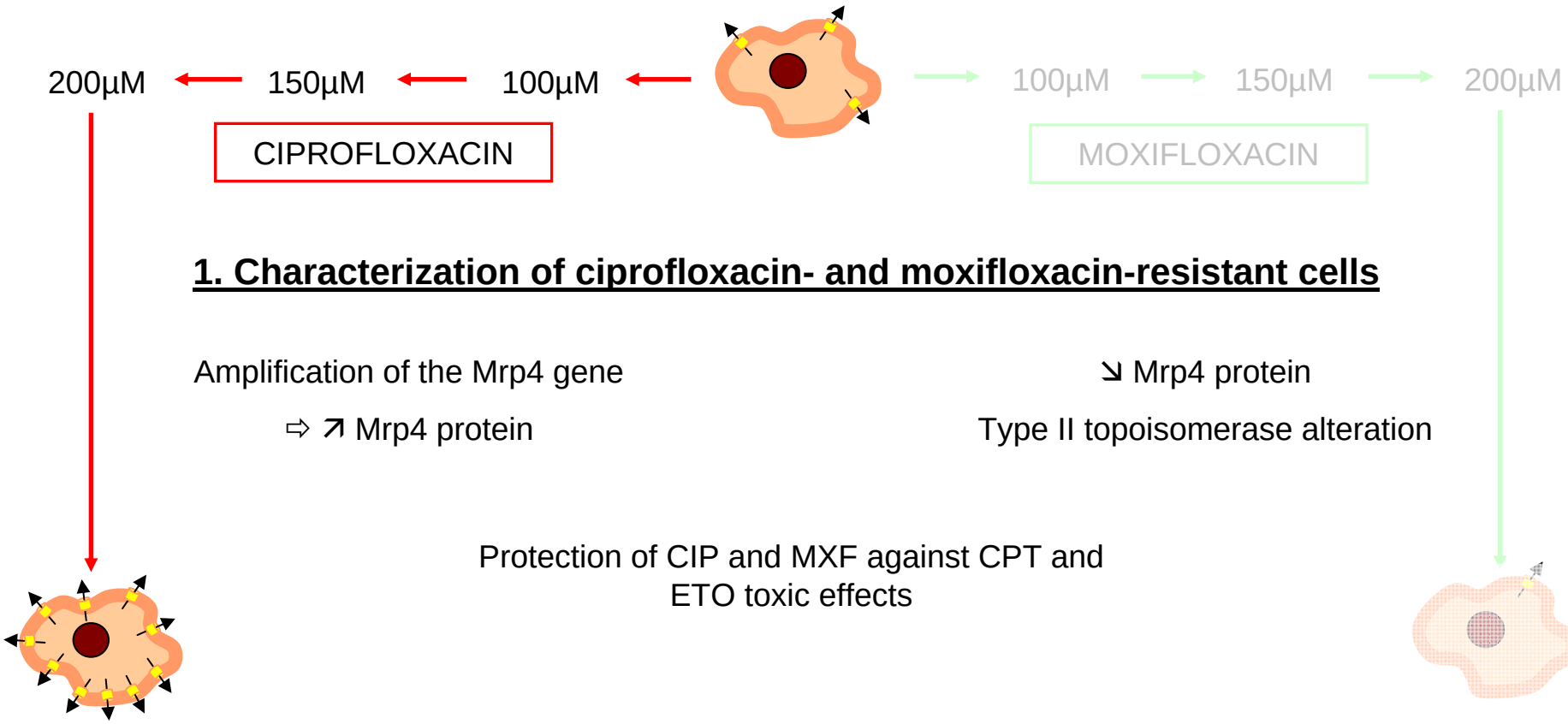


1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells

Amplification of the Mrp4 gene

⇨ ↗ Mrp4 protein

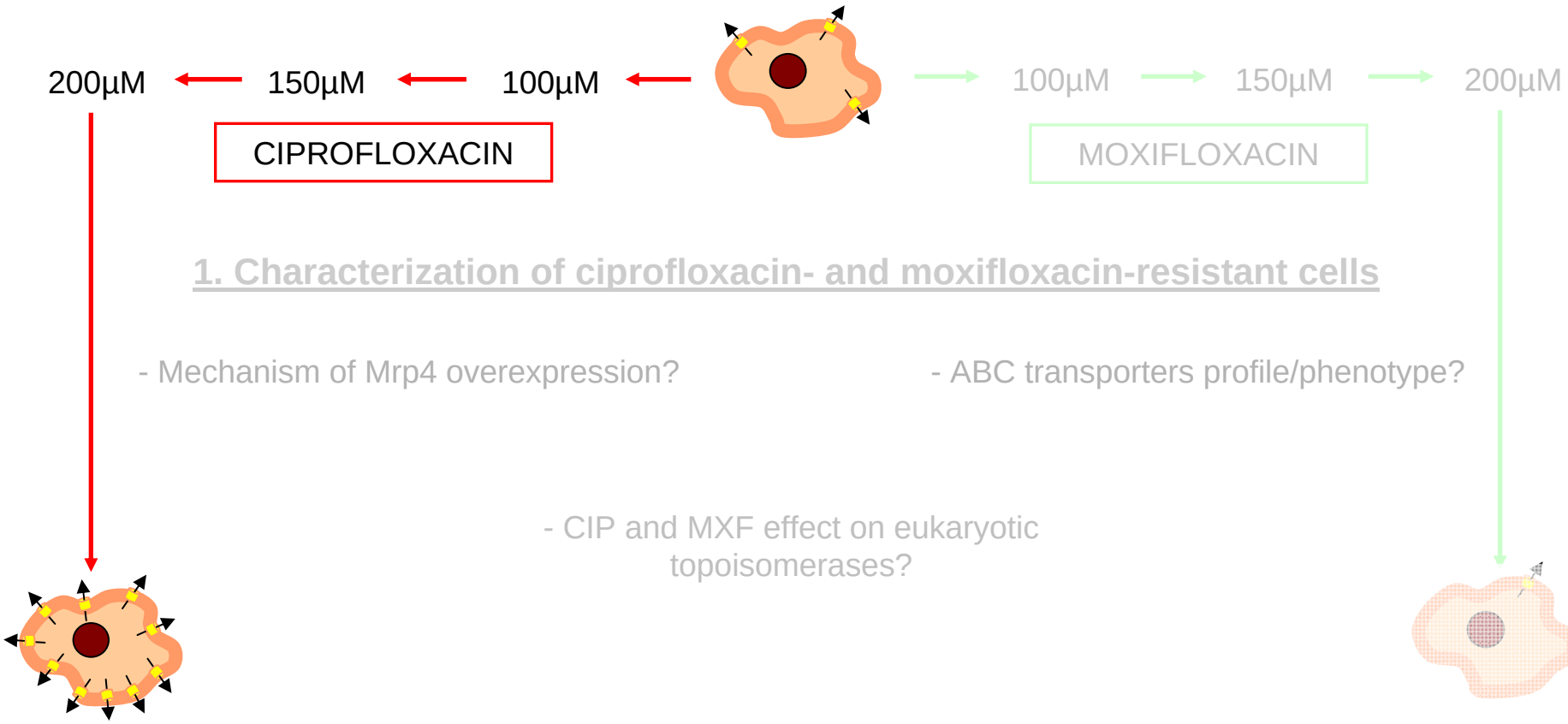
↘ Mrp4 protein

Type II topoisomerase alteration

Protection of CIP and MXF against CPT and ETO toxic effects

2. Mrp4: fluoroquinolone recognition and transport

SUMMARY



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International Journal of Antimicrobial Agents 38 (2011) 249–256

Contents lists available at ScienceDirect

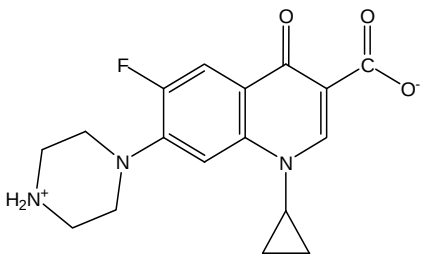
International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

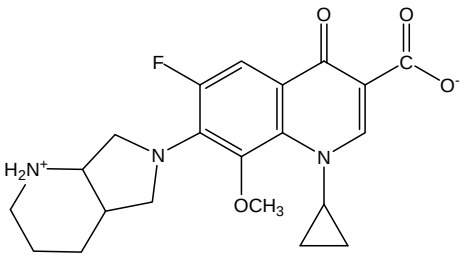
Cellular accumulation of fluoroquinolones is not predictive of their intracellular activity: studies with gemifloxacin, moxifloxacin and ciprofloxacin in a pharmacokinetic/pharmacodynamic model of uninfected and infected macrophages

Coralie M. Vallet, Béatrice Marquez[1], Eva Ngabirano, Sandrine Lemaire, Marie-Paule Mingeot-Leclercq, Paul M. Tulkens*, Françoise Van Bambeke

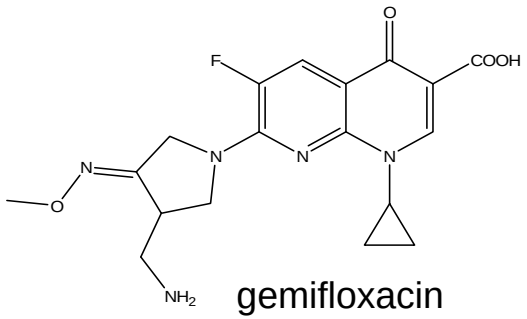
Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Avenue E. Mounier 73 bte B1.73.05, B-1200 Brussels, Belgium



ciprofloxacin



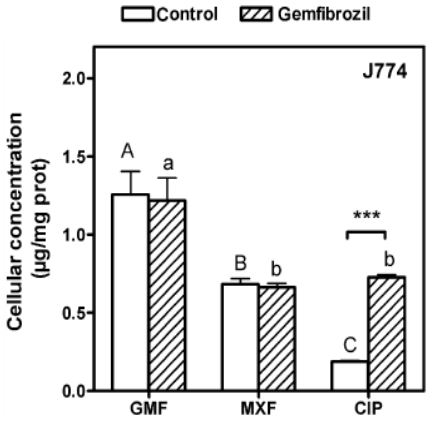
moxifloxacin



gemifloxacin

RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation



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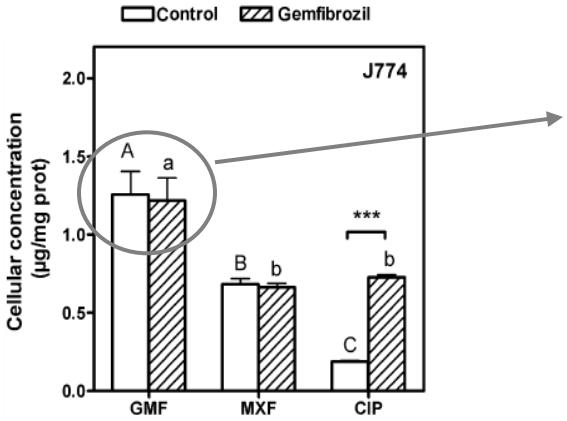

⇨High gemifloxacin accumulation level

⇨As MXF, GMF accumulation is not affected by an Mrp transporter in WT cells

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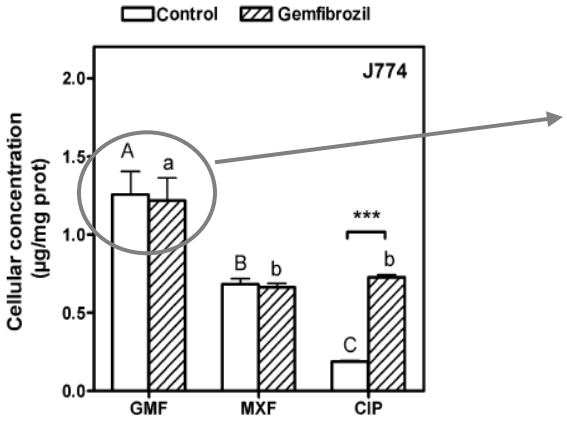

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Has GMF a higher intracellular activity?

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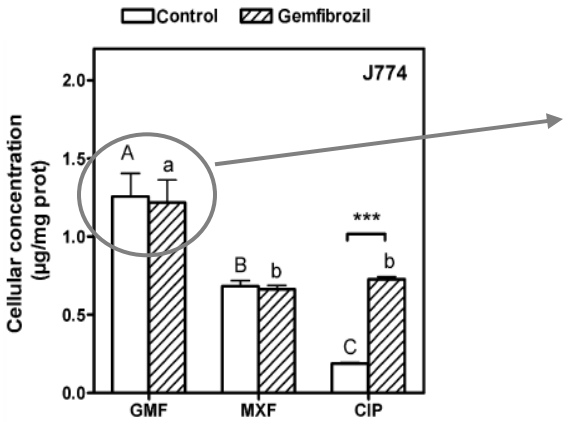

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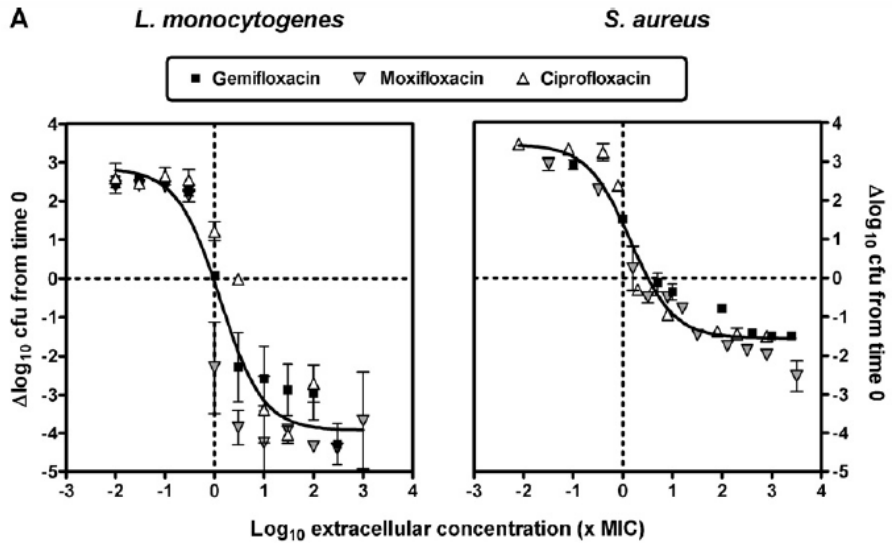
Intracellular activity of GMF, MXF and CIP against *S. aureus* and *L. monocytogenes*



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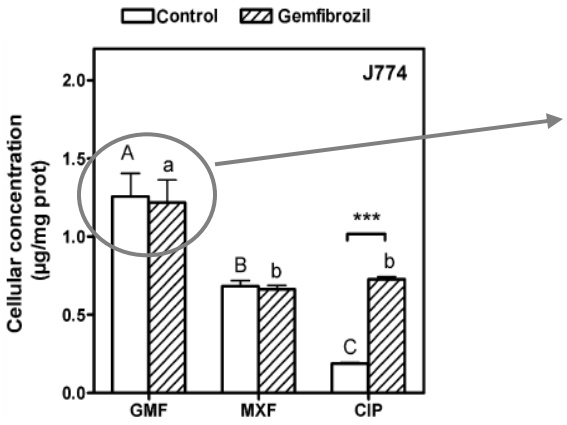

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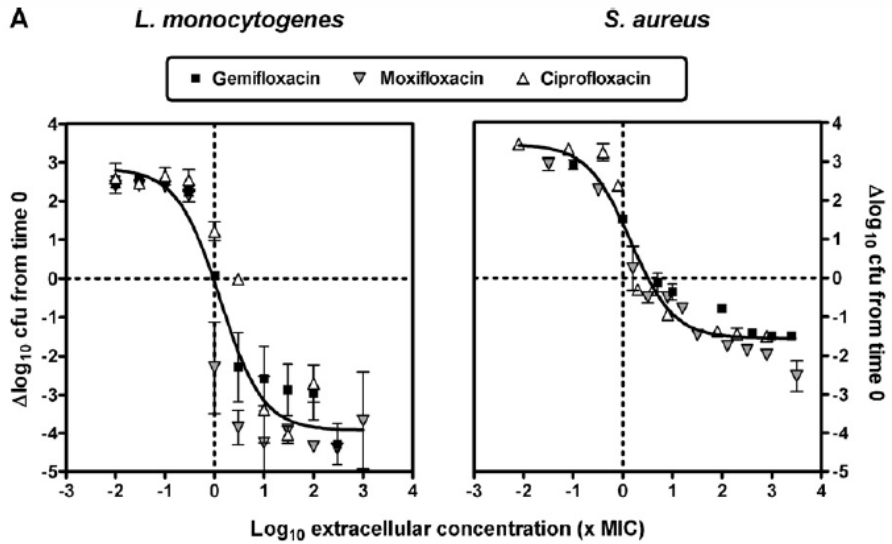

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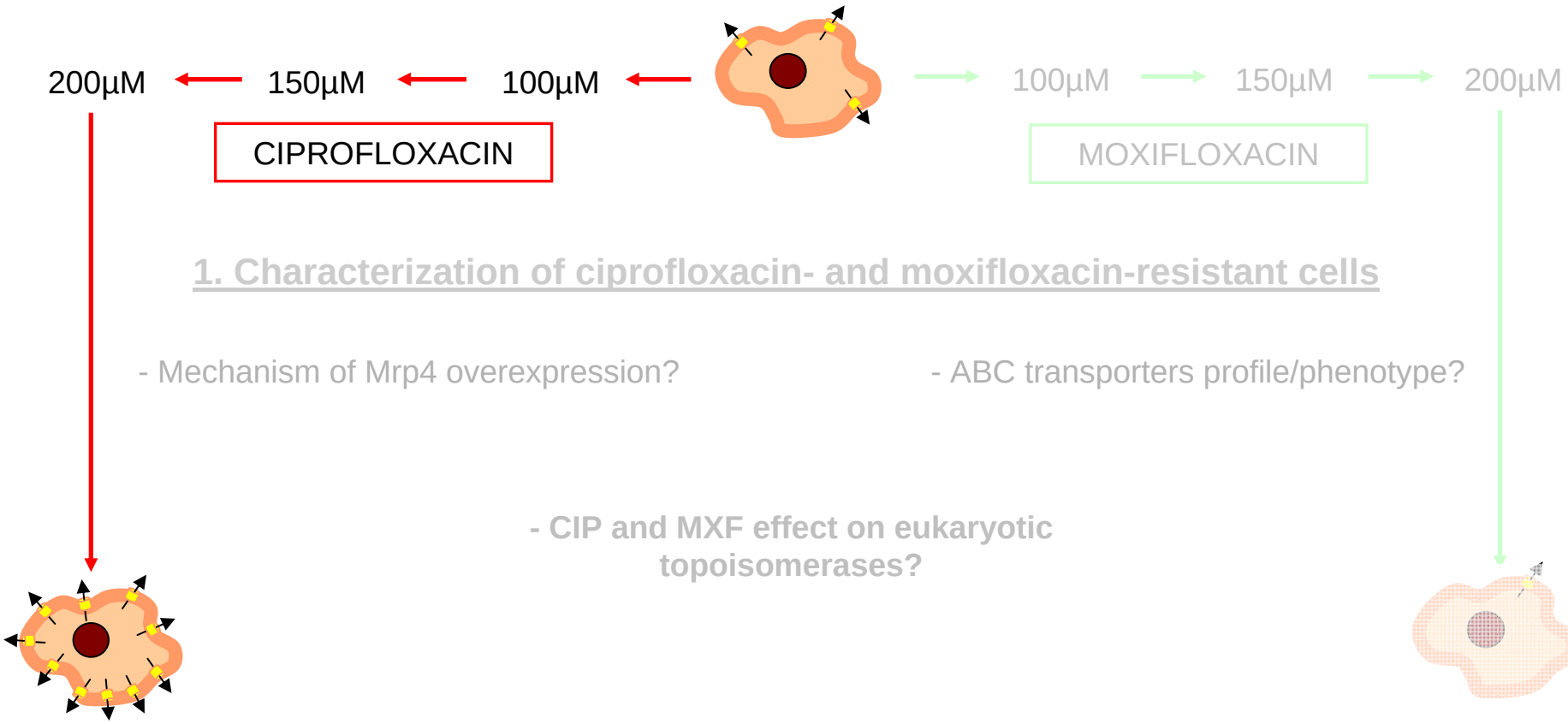
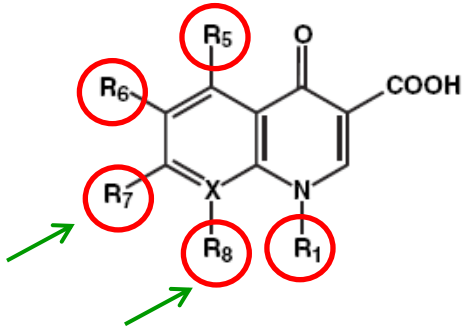


2. Mrp4: fluoroquinolone recognition and transport

Molecular determinants for recognition by an efflux pump?

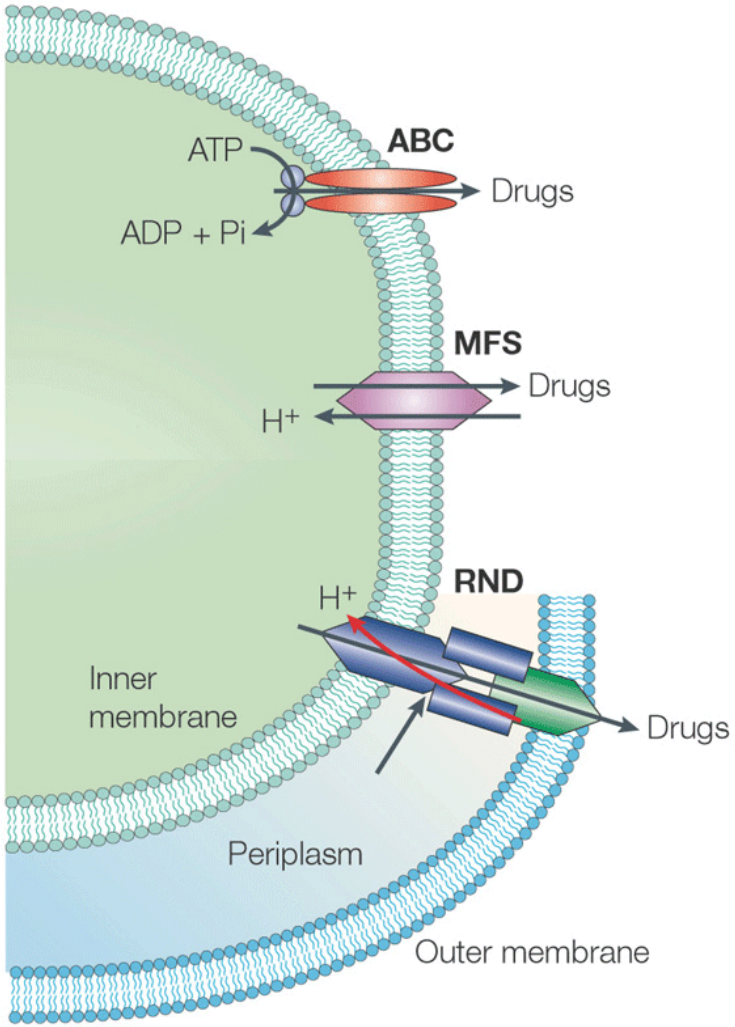
Substrate recognition by efflux pumps in prokaryotes (NorA in *S. aureus*, PatA/PatB in *S. pneumoniae*, Mex/Opr in *P. aeruginosa*) and in eukaryotes (Mrp4 in murine J774 macrophages): a combined biological and structural study with 25 fluoroquinolones

(The structural study was performed in collaboration with Martine Prévost and Julien Dupont, *Structure et Fonction des Membranes biologiques*, Université Libre de Bruxelles)



RESULTS II: fluoroquinolone structure and PK profile

Bacteria efflux pumps



PatA/PatB from *Streptococcus pneumoniae*

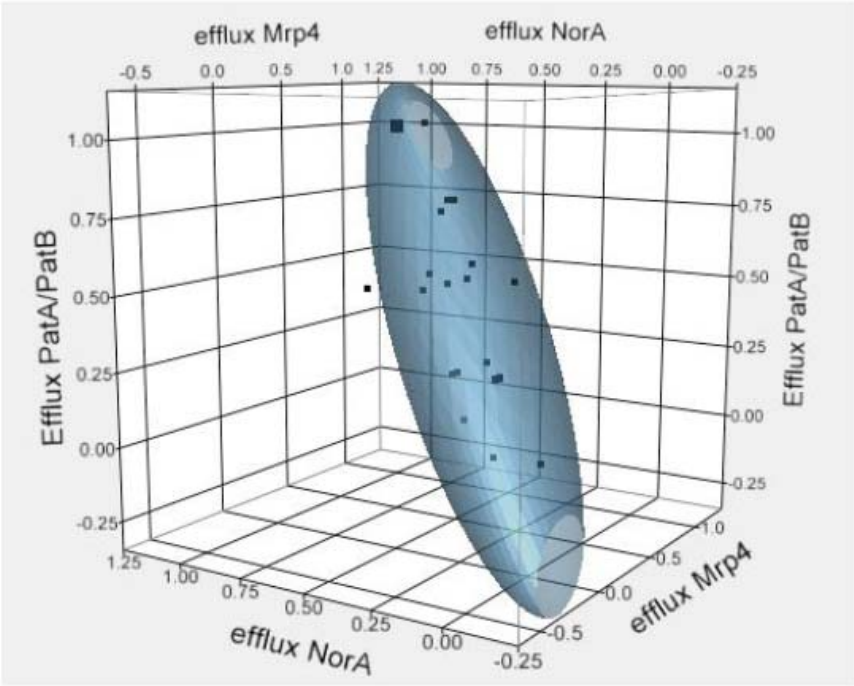
NorA from *Staphylococcus aureus*

Mex/Opr system from *Pseudomonas aeruginosa*

RESULTS II: fluoroquinolone structure and PK profile

Bacteria *vs* mouse macrophages:

Δ accumulation levels maximal efflux (CIP-R) and minimal efflux (WT + Gem)



eukaryotes / Gram+ ⇨ good correlation

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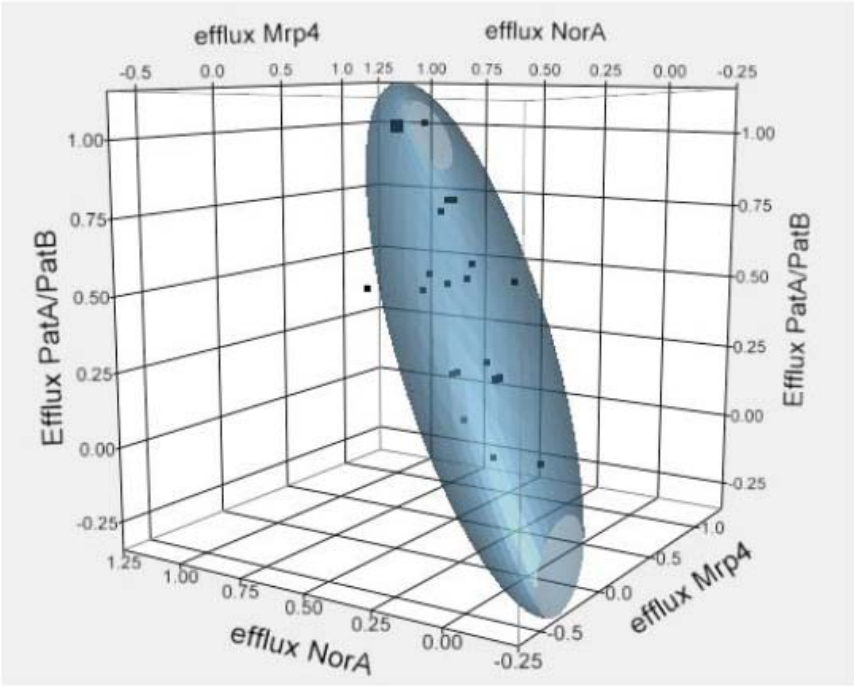

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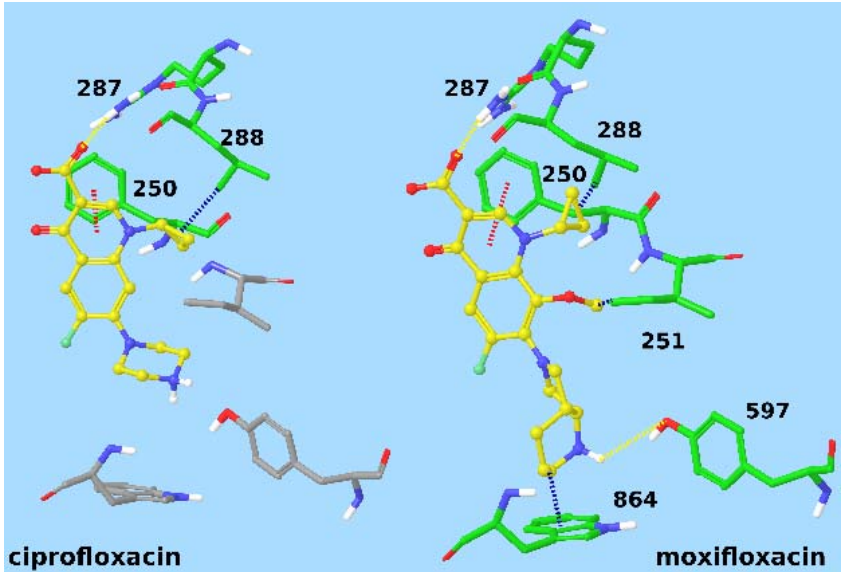
Is there one physicochemical parameter which govern the sensitivity to efflux by Mrp4, NorA and PatA/PatB?

⇨ NO

RESULTS II: fluoroquinolone structure and PK profile

Interactions between fluoroquinolones and Mrp4



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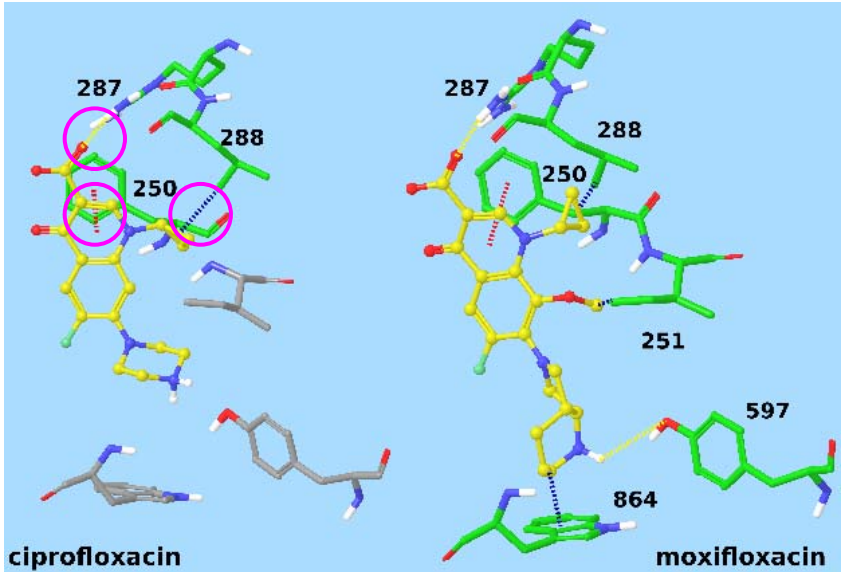

CIP: 3 interactions with Mrp4

RESULTS II: fluoroquinolone structure and PK profile

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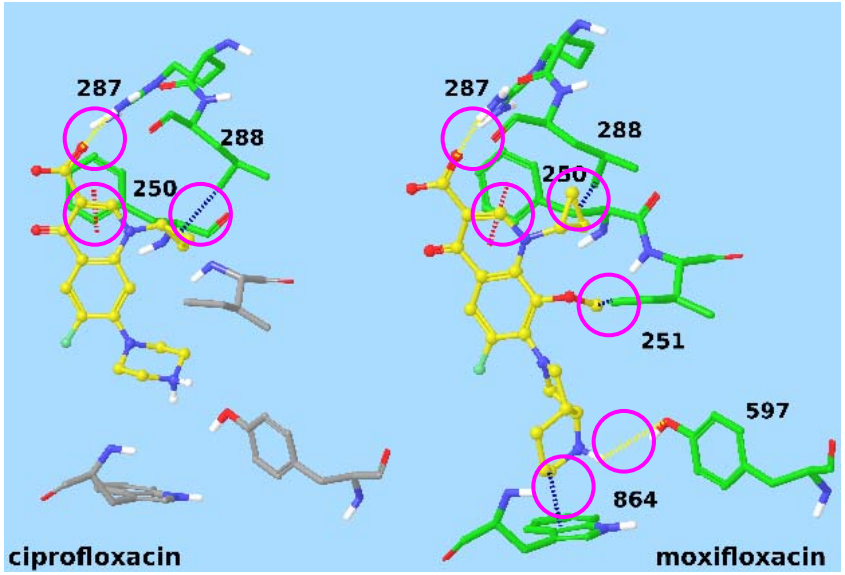

CIP: 3 interactions with Mrp4

MXF: **6** interactions with Mrp4

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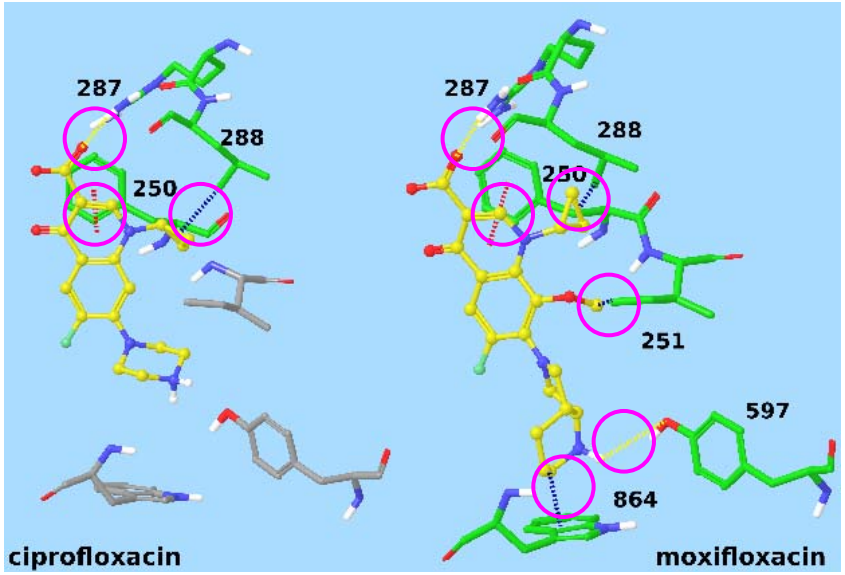

CIP: 3 interactions with Mrp4

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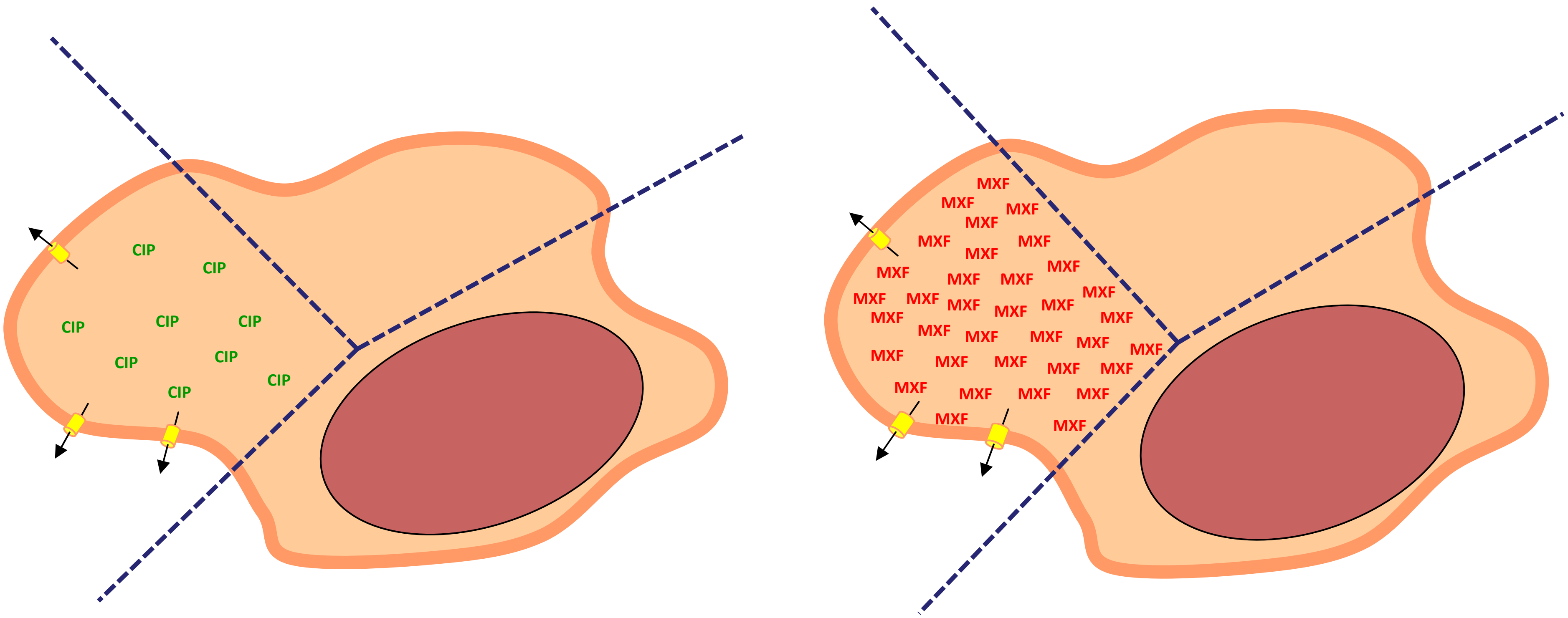
Efflux ⇨ less interactions

No efflux ⇨ more interactions

TAKE HOME MESSAGE

✓ 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**



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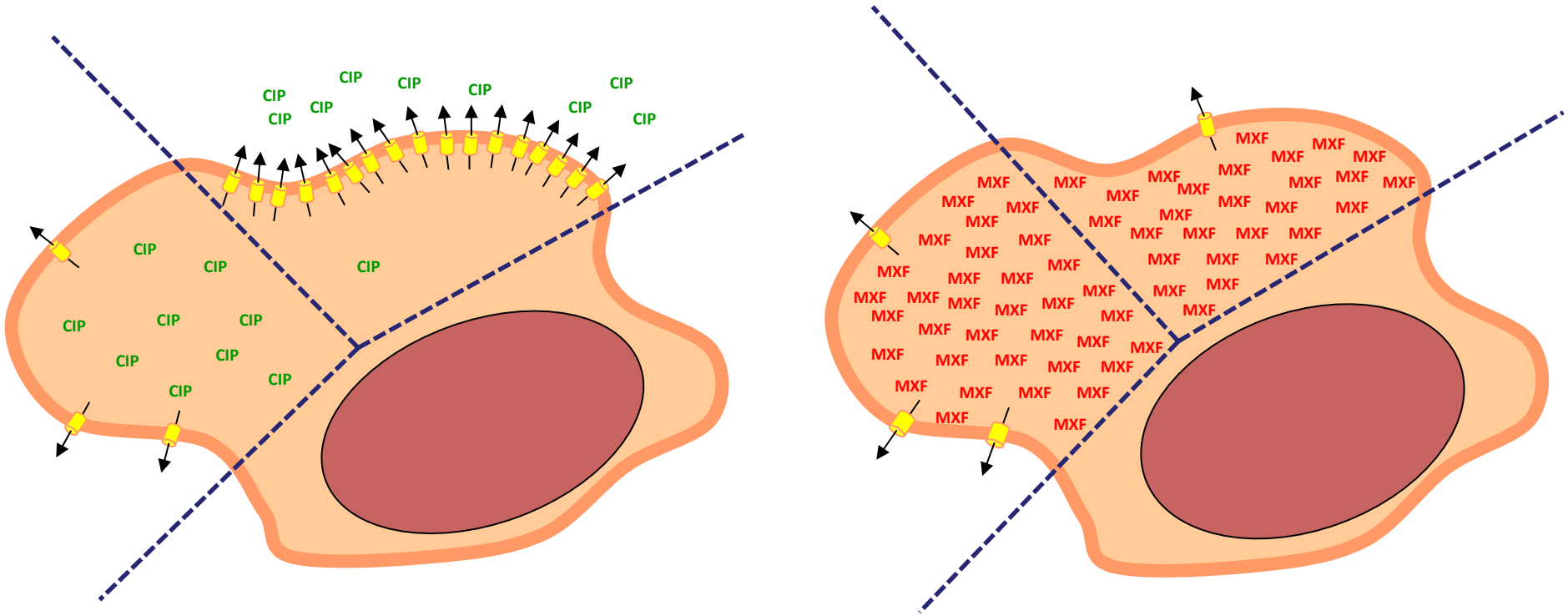

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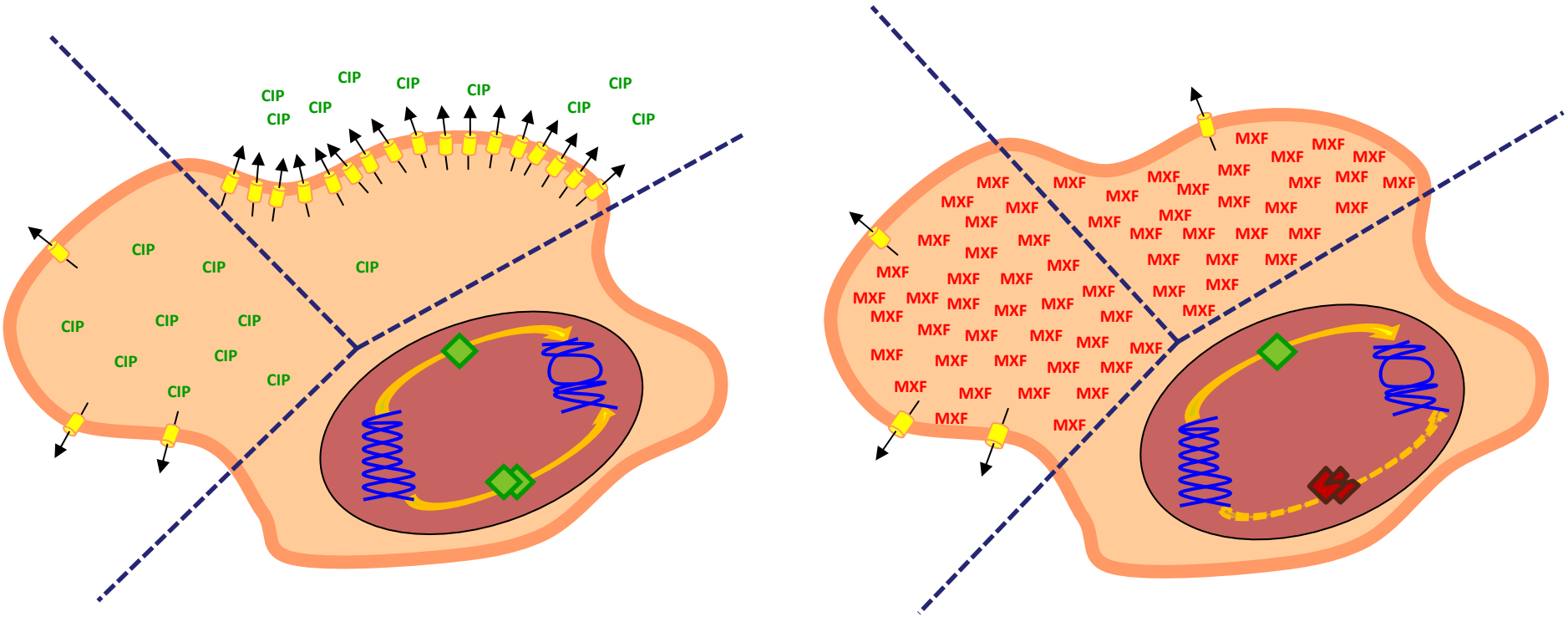

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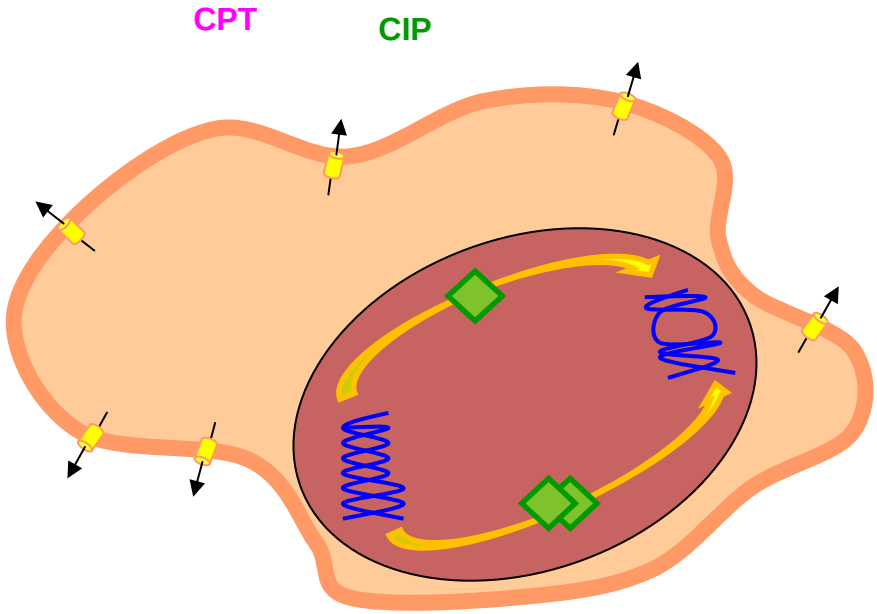

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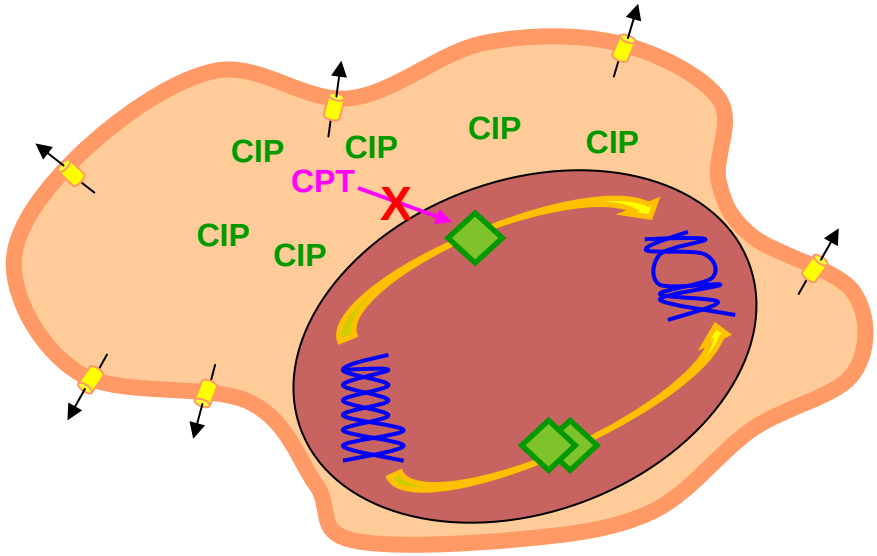

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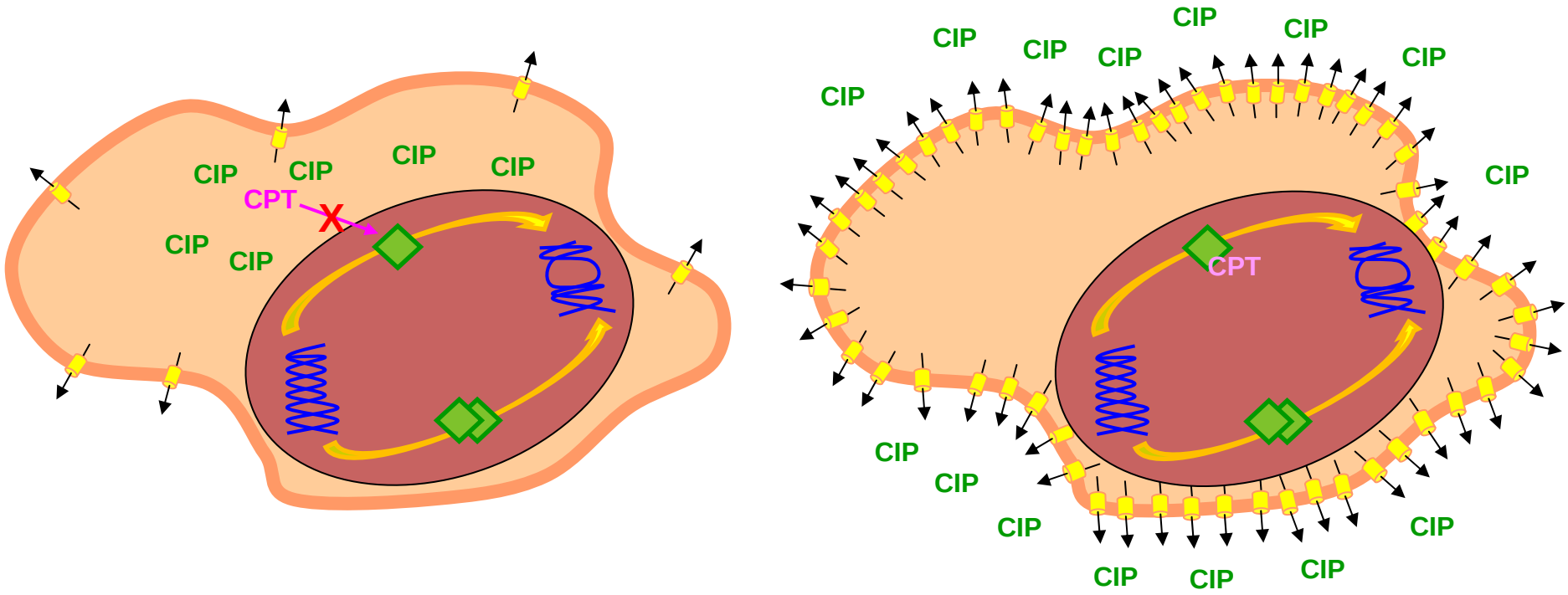

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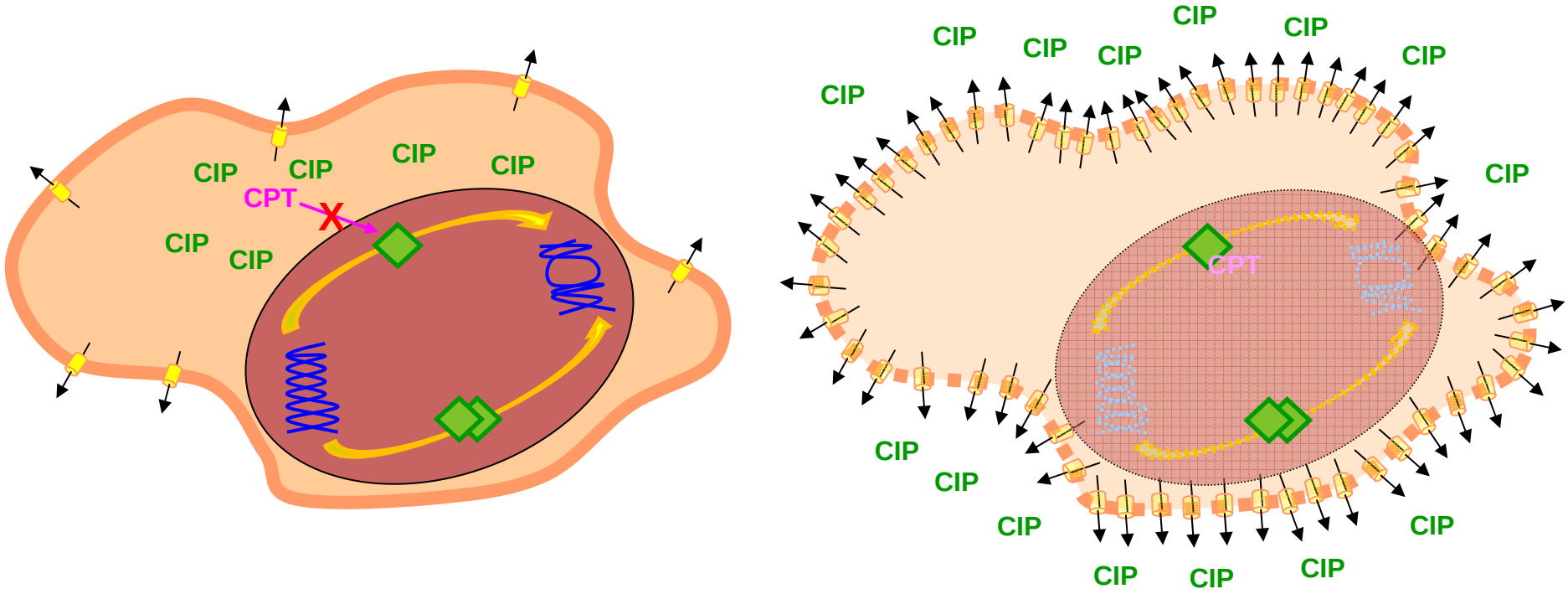

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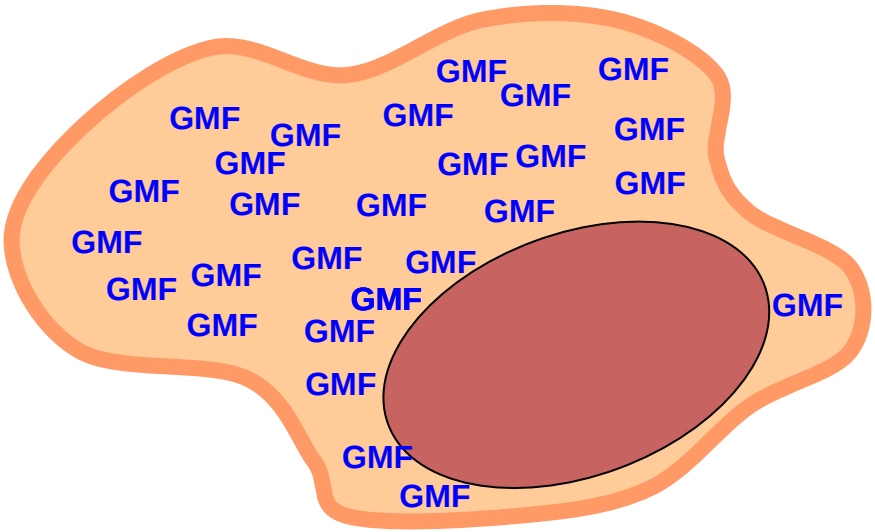

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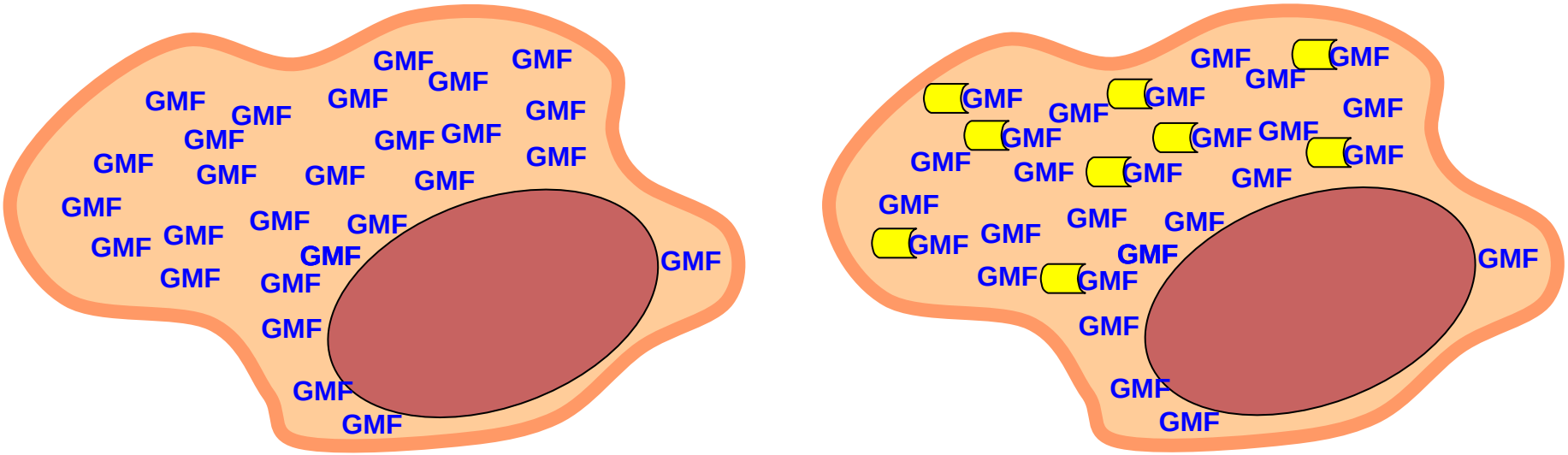

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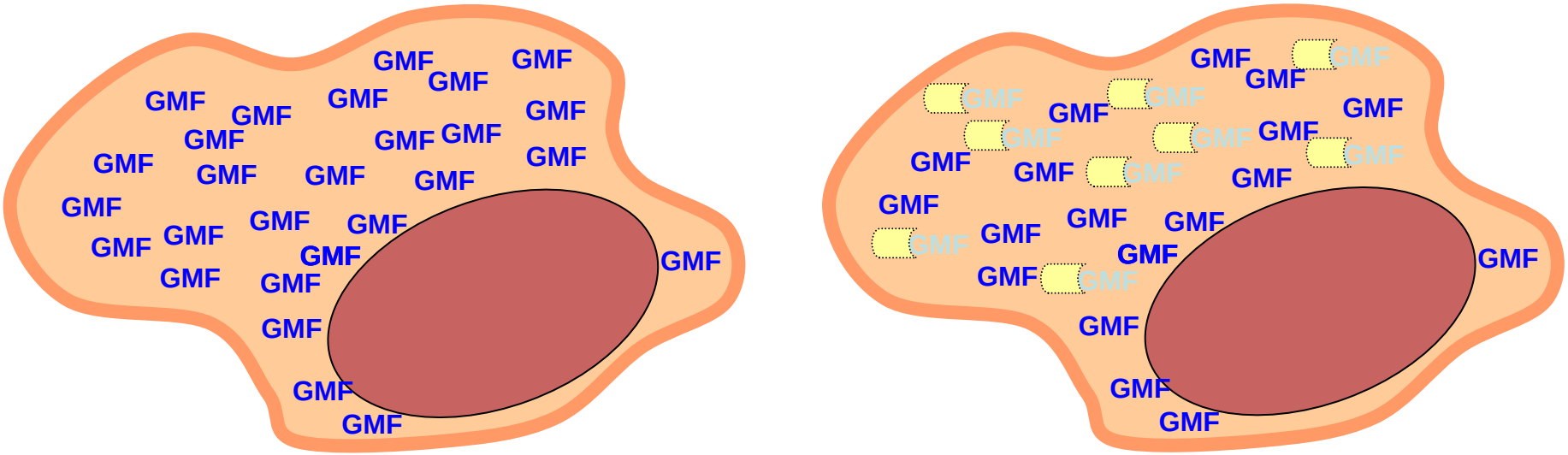

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✓ All **fluoroquinolones** are "**substrates**" of efflux pumps

⇨ Efflux is linked to the **number of interactions** the molecule does with the binding site of the efflux pump

PERSPECTIVES

❖ All fluoroquinolones are recognized by the Mrp4 efflux pump, but following their sensitivity to efflux, the resistance mechanism in cells exposed to FQs can differ.

What else now??

✓ MXF-R cells : what is the mechanim leading to Mrp4 reduction in expression ?
type II topoisomerase = resistance mechanism?

✓ How do fluoroquinolones protect cells against anticancer agents toxic effects?

✓ Intracellular bioavailability of fluoroquinolones?

✓ Transport of fluoroquinolones by Mrp4 : Mrp4 cristal strucutre?

THANKS TO …

- Françoise Van Bambeke
- Prof. P. Tulkens, M-P Mingeot-Leclercq
- Members of the jury
- Collaborators: Martine Prévost and Julien Dupont (ULB), Dr. Wetzstein (Bayer)
- F.S.R. and F.R.I.A.
- My family

THANKS TO …

All old and new FACMists… My colleagues and friends