

**Ehrlich II - 2<sup>nd</sup> World Conference on Magic Bullets**

Nuremberg October 3-5, 2008

# **Can We Make Eucaryotic Cells Resistant to Antibiotics?**

## **Correlation between Multidrug Resistance-associated Protein (Mrp) Efflux Pump Expression and Fluoroquinolones Accumulation in J774 Macrophages**

**B. MARQUEZ, C. VALLET, M-P. MINGEOT-LECLERCQ,  
P.M. TULKENS, F. VAN BAMBEKE**

Unité de Pharmacologie cellulaire et moléculaire - Université catholique de Louvain

Brussels, Belgium



# Efflux pumps

⇒ **UBIQUITOUS** mechanism of resistance to **DRUGS**

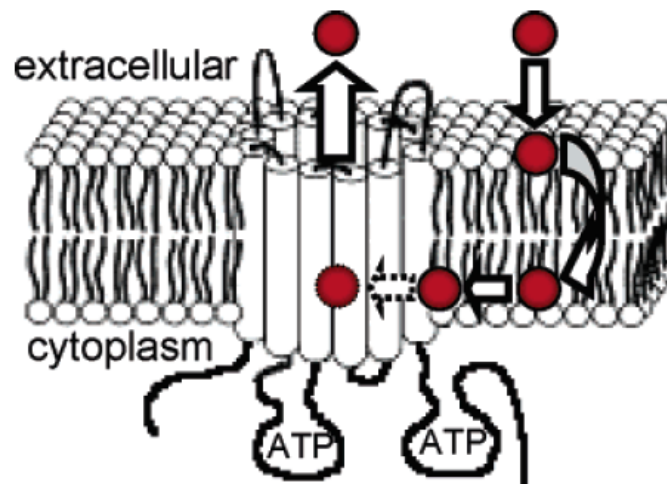
✓ **Bacteria:** resistance to **ANTIBIOTICS**

mediated mainly by secondary transporters

✓ **Eucaryotes:** resistance to **ANTICANCER**, antifungal drugs...

mediated mainly by ATP-Binding Cassette (ABC) transporters

↳ P-glycoprotein, MRPs (MRP1, MRP2..) and BCRP



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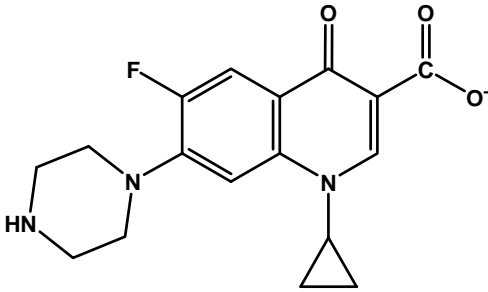
**BUT ABC transporters also expel antibiotics!**

	fluoroquinolones	cephalosporins	penicillins	macrolides	tetracyclines
P-gp	+	-	-	+	+
MRPs	+	+	+	+	+
BCRP	+	+ (rat)	-	+	-

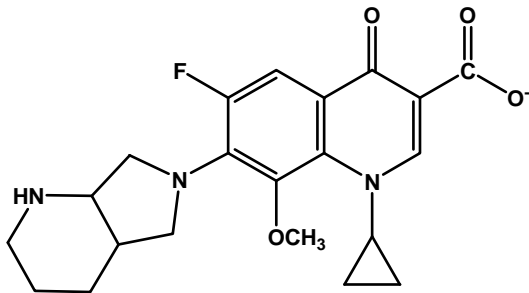
# Two fluoroquinolones...

Measure of FQ accumulation and efflux in murine J774 **wild-type** macrophages

## Ciprofloxacin (CIP)



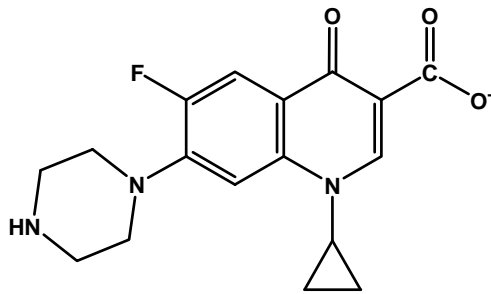
## Moxifloxacin (MXF)



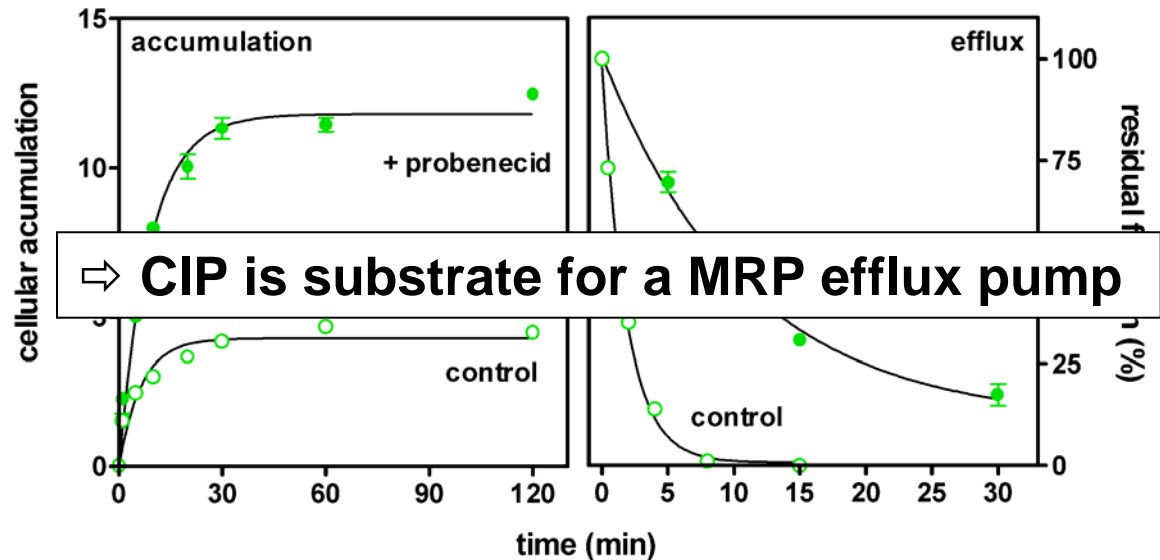
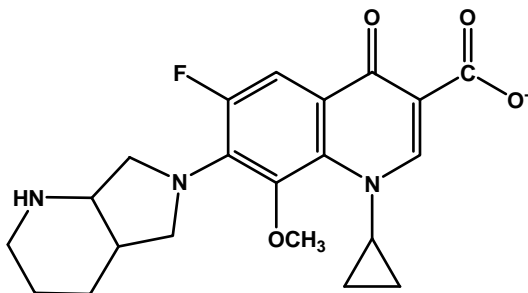
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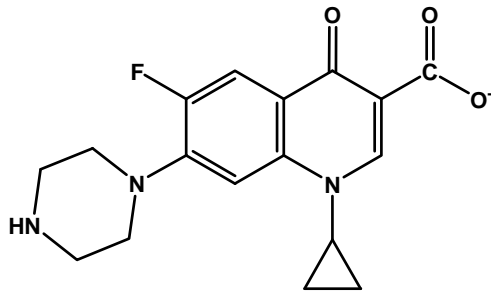
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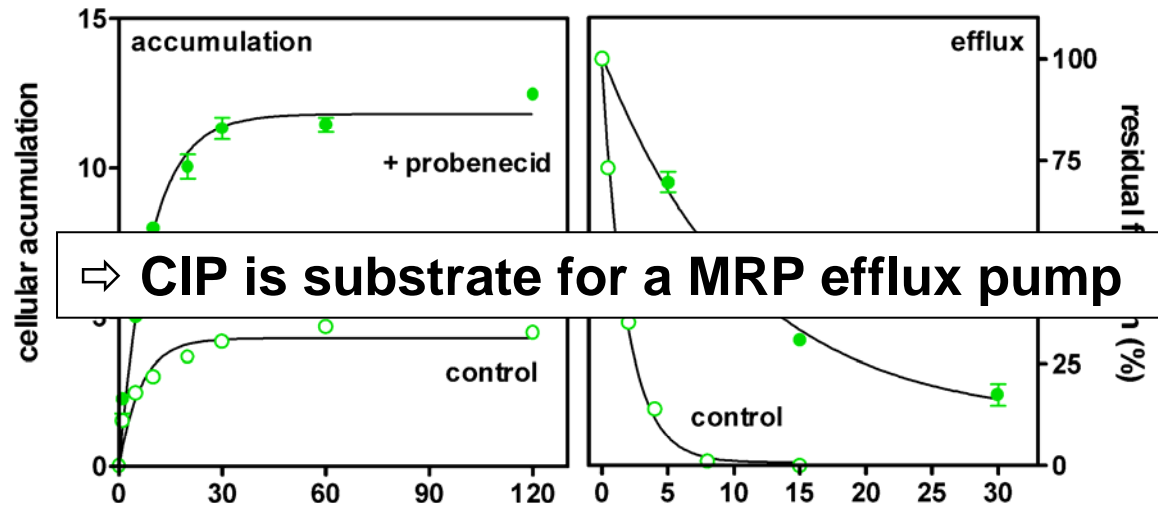
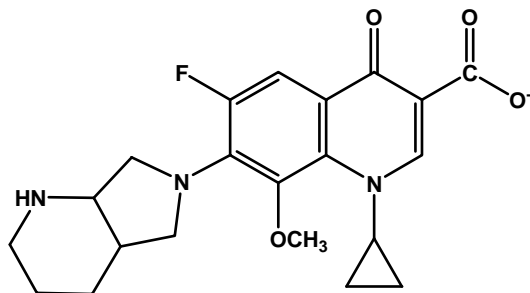
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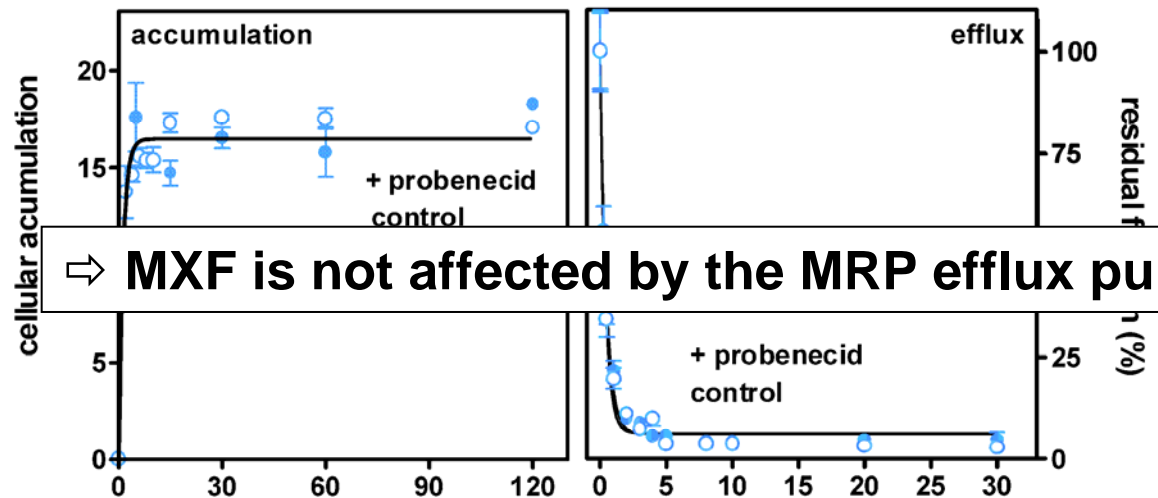
## Ciprofloxacin (CIP)



## Moxifloxacin (MXF)



⇒ **CIP is substrate for a MRP efflux pump**



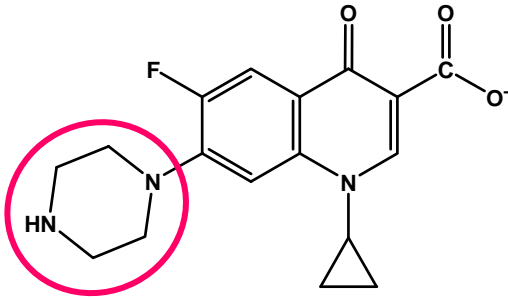
⇒ **MXF is not affected by the MRP efflux pump**

time (min)

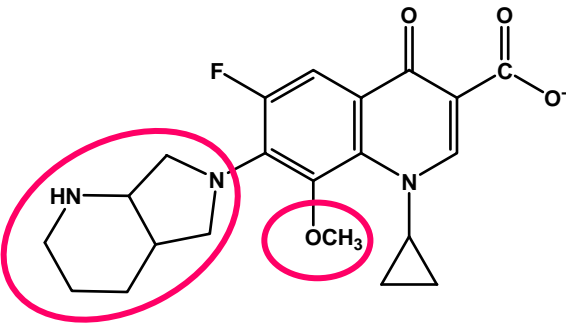
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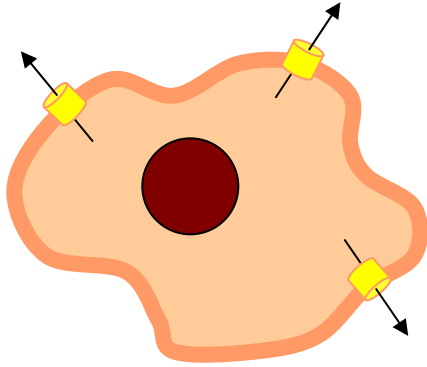
## Moxifloxacin (MXF)



... with a different behavior with respect to transport by MRPs

# Aim of the study

Can we select macrophages resistant to CIP or MXF?

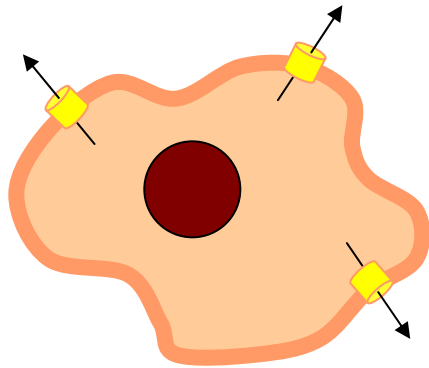


WT macrophages with a MRP pump for CIP



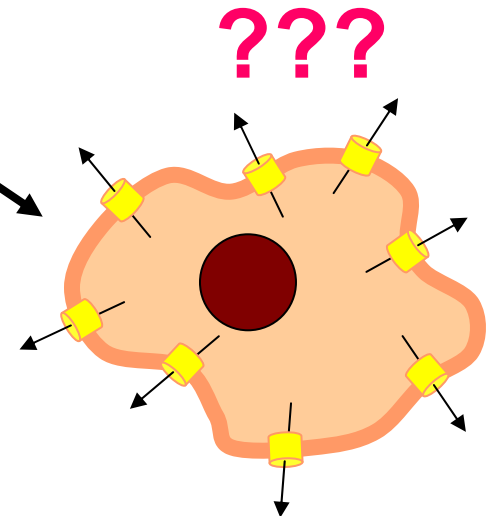
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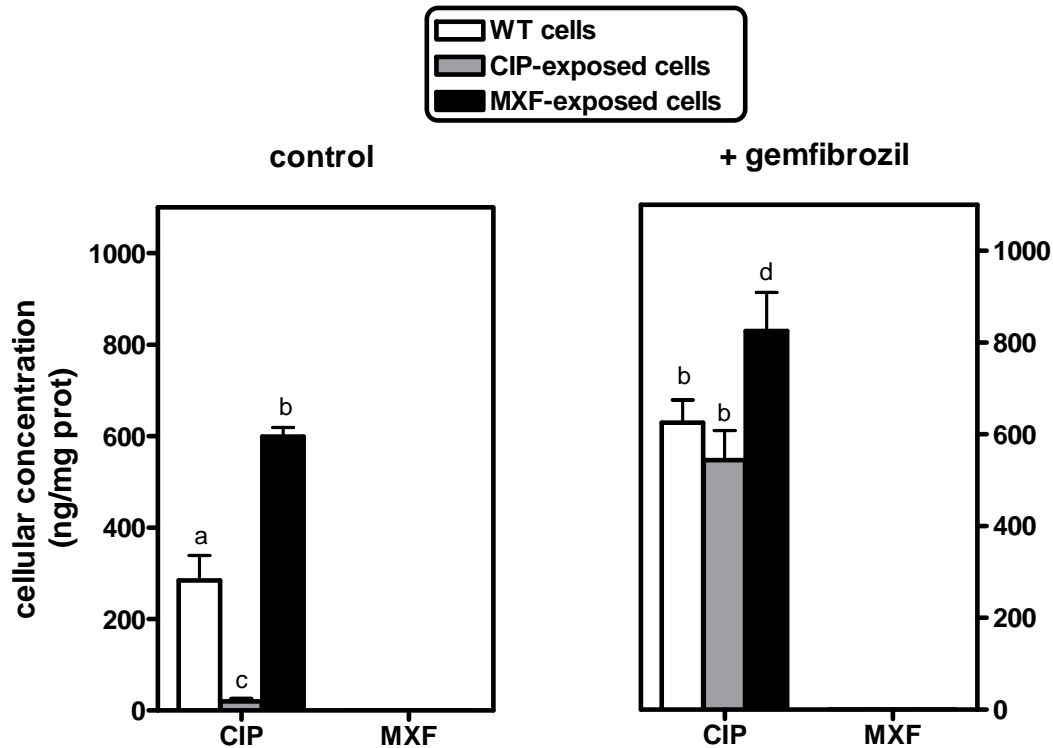
WT macrophages with a MRP pump for CIP

**Chronic exposure to increasing concentrations of CIP or MXF**



Selected macrophages: resistant to CIP or MXF?

# Fluoroquinolones accumulation

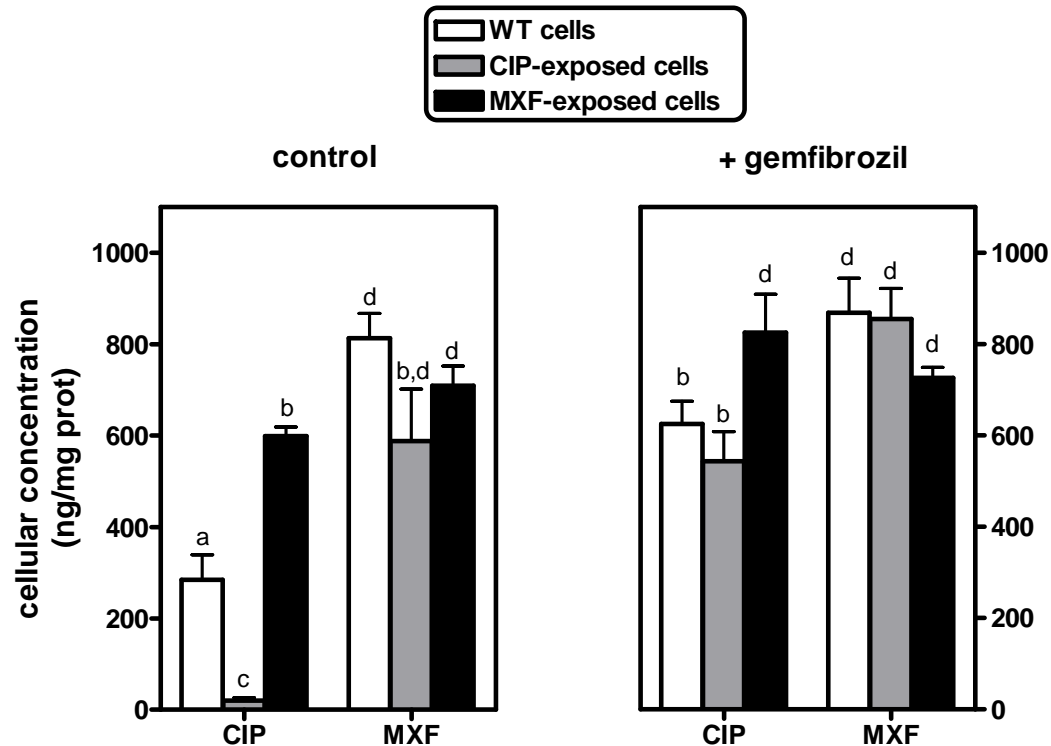


**CIP accumulation** varies between the 3 cell lines:

↘ in **CIP-exposed macrophages** ⇒ **resistant** macrophages

↗ in **MXF-exposed macrophages**

# Fluoroquinolones accumulation



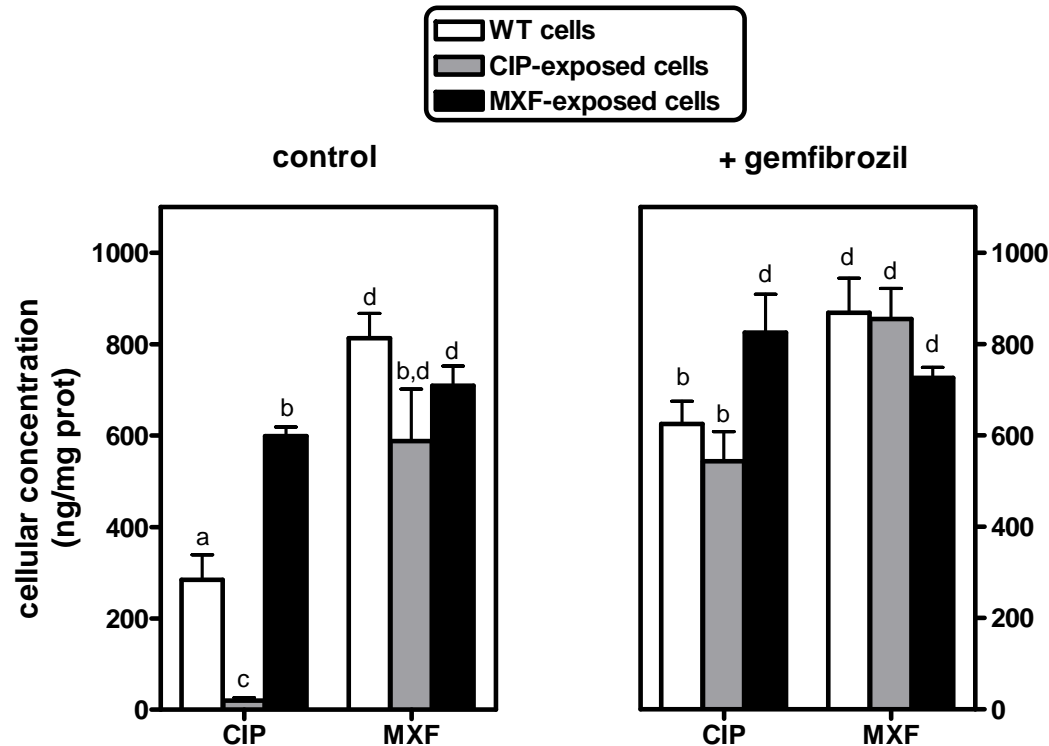
**CIP accumulation** varies between the 3 cell lines:

↘ in **CIP-exposed macrophages** ⇒ **resistant** macrophages

↗ in **MXF-exposed macrophages**

**MXF accumulation** does not change significantly among the 3 cell lines

# Fluoroquinolones accumulation



↪ **CIP** selects for macrophages resistant to CIP (lower CIP accumulation) but not to MXF

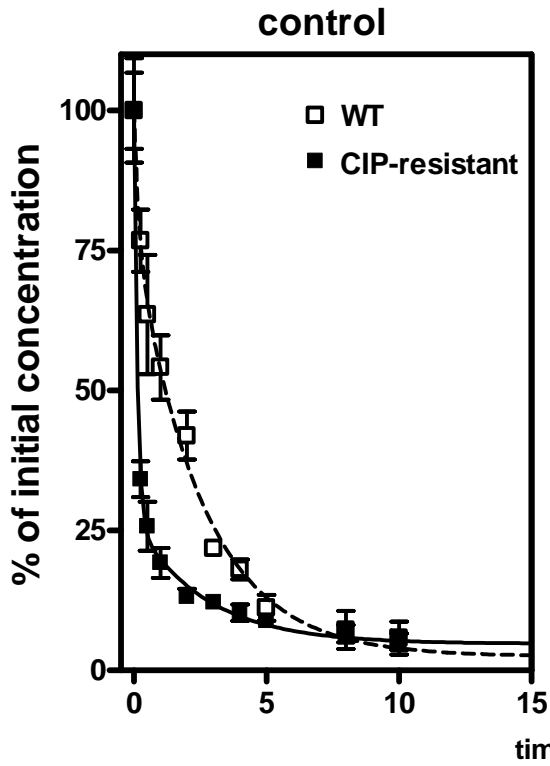
↪ **MXF** does not select for macrophages resistant to MXF

↪ Exposure to **MXF** induces an increase of CIP accumulation in macrophages

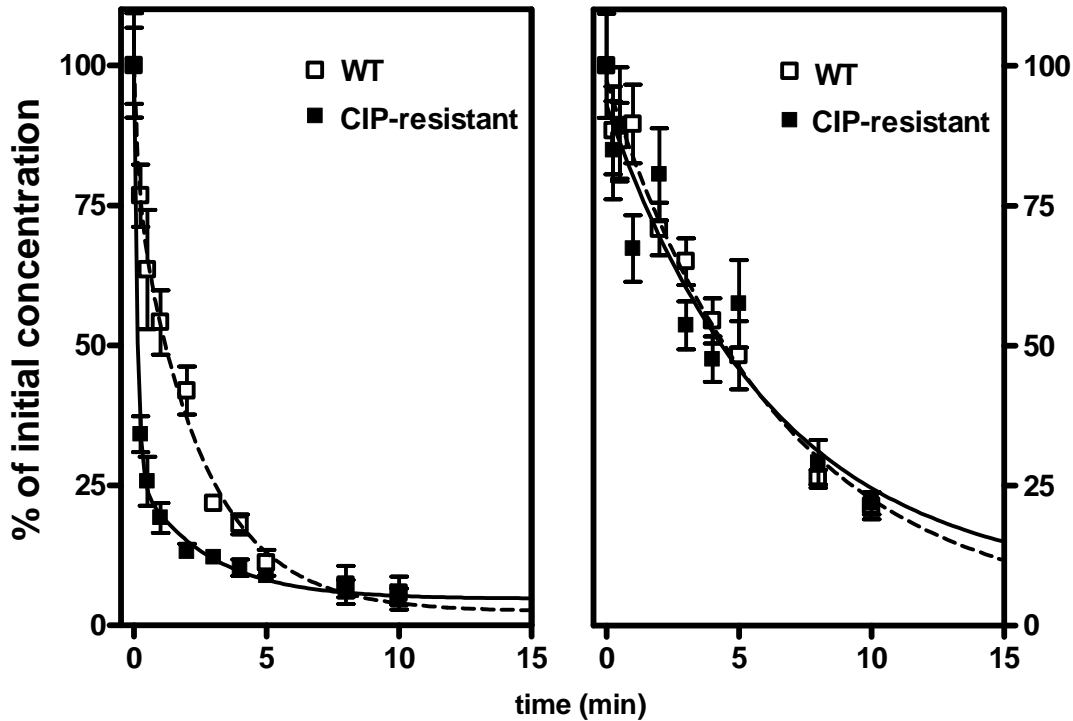
# CIP kinetics

## CIP efflux

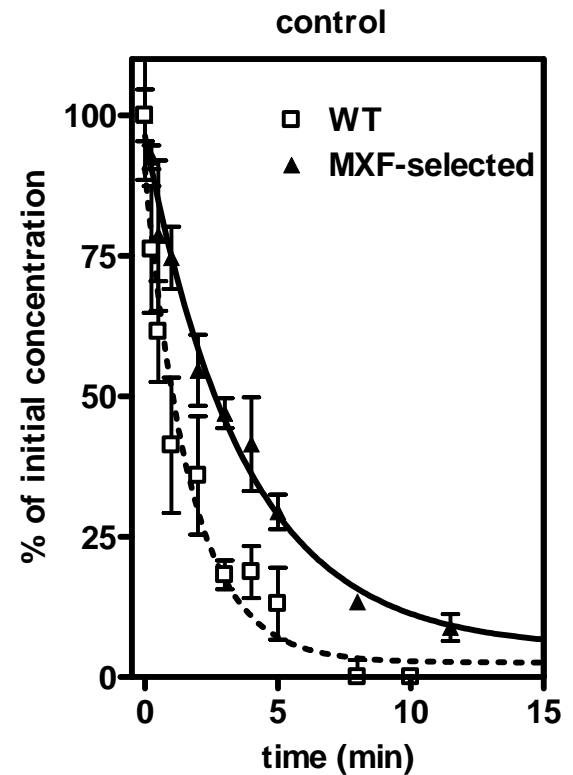
In CIP-resistant macrophages



+ gemfibrozil



In MXF-selected macrophages

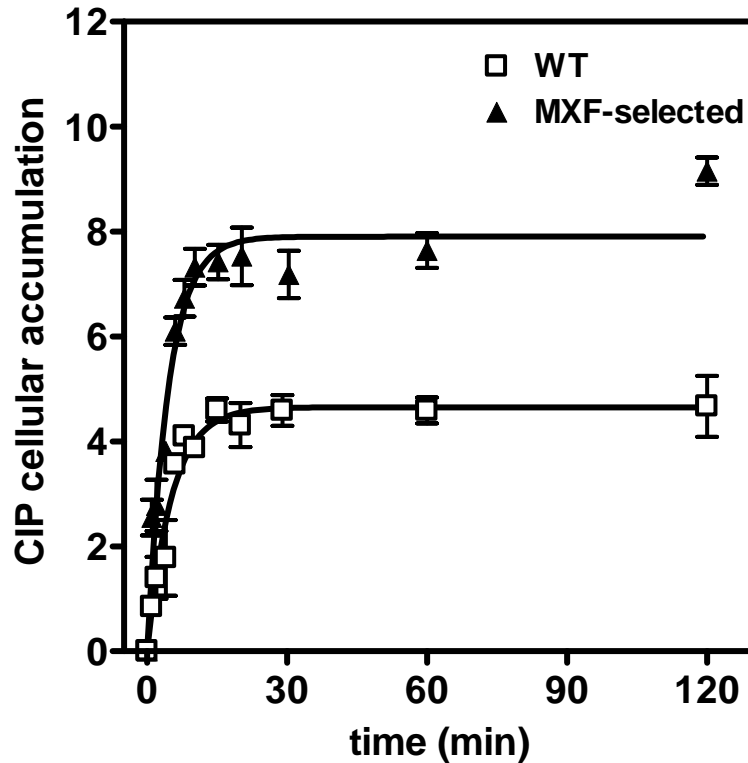


**CIP efflux  $T_{1/2}$ : CIP-resistant < WT < MXF-selected macrophages**

# CIP kinetics

## CIP influx

In MXF-selected macrophages



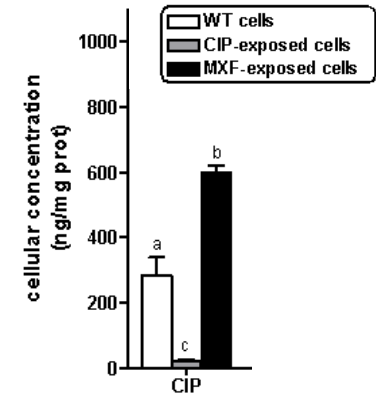
⇒ Different levels of CIP accumulation but similar  $T_{1/2}$

In CIP-resistant macrophages: not possible to measure (level of accumulation too low)

# CIP kinetics

## $T_{1/2}$ for CIP

	Influx	Efflux
<b>WT macrophages</b>	3.6 min	1.2 min
<b>CIP-resistant</b>	nd	< 0.1 min
<b>MXF-selected</b>	3.1 min	2.6 min



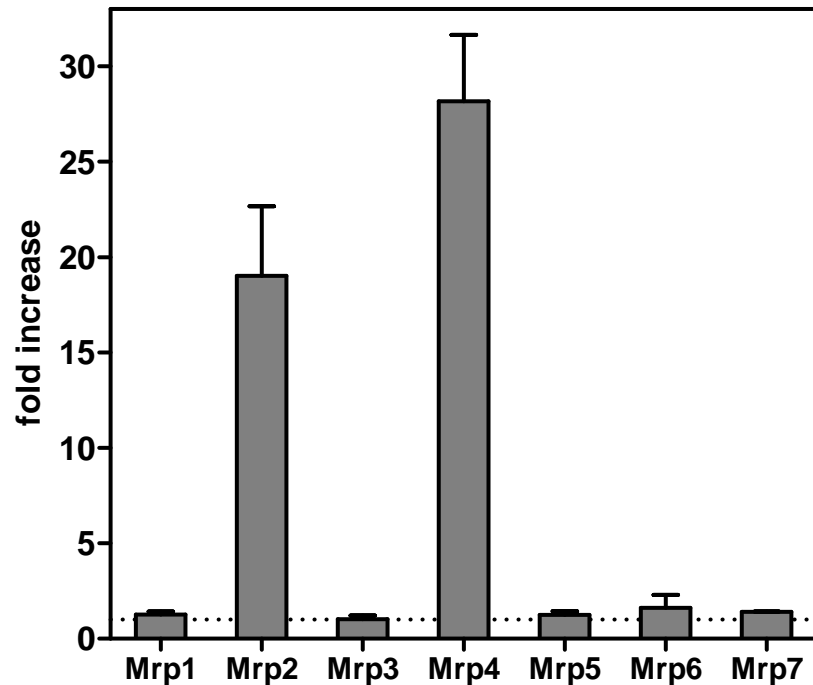
⇒ **Differences in CIP accumulation** are the consequences of a **faster (CIP-resistant macrophages)** or **slower (MXF-selected macrophages) efflux** of the drug.

☞ Which MRP efflux pump is involved?

# MRP content

- ◆ Analysis at the mRNA level by real-time PCR

In CIP-resistant macrophages



	<i>Mrp4</i> expression ratio
<b>WT macrophages</b>	1
<b>CIP-resistant</b>	28
<b>MXF-selected</b>	0.3

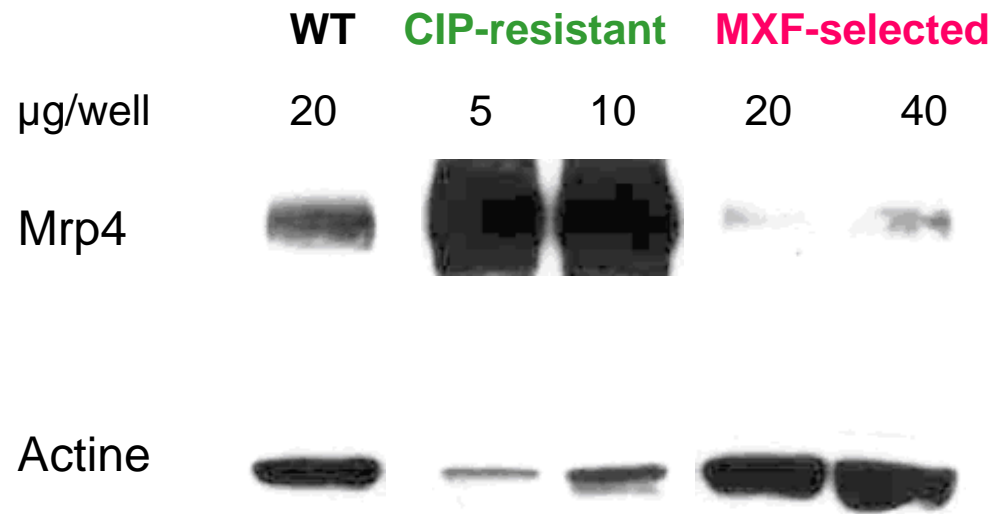
⇒ Overexpression of *Mrp2* and *Mrp4* in CIP-resistant macrophages

⇒ Lower expression of *Mrp4* in MXF-selected macrophages



# MRP content

- ◆ Analysis at the protein level by Western-Blot



⇒ CIP-resistant macrophages express more Mrp4

⇒ MXF-selected macrophages express less Mrp4

# Conclusions

- ✓ **Fluoroquinolones are differentially affected by MRP efflux pumps in WT macrophages.**
- ✓ **CIP can select for resistant macrophages (by overexpression of efflux pumps) whereas MXF cannot.**
- ✓ **Chronic exposure to CIP or MXF selects for opposite phenotypes (different level of expression of Mrp4).**
- ✓ **CIP accumulation is inversely correlated with Mrp4 expression.**