

Colloque ABC franco-belge (6ème journée)

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Active transport of ciprofloxacin in murine macrophages

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Brussels, Belgium



Efflux pumps

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

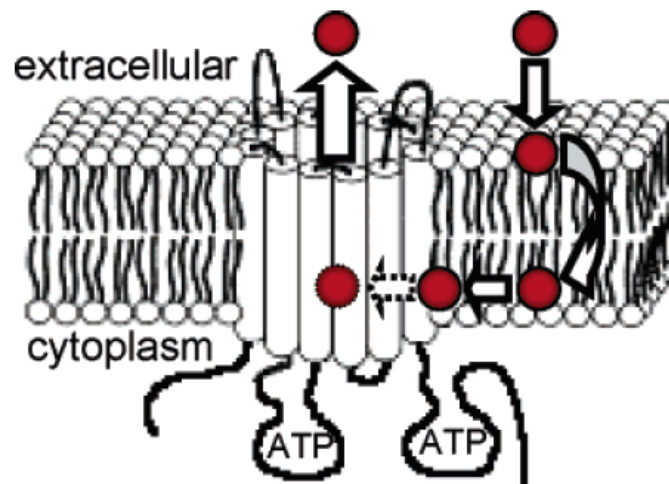
✓ **Bacteria:** resistance to **ANTIBIOTICS**

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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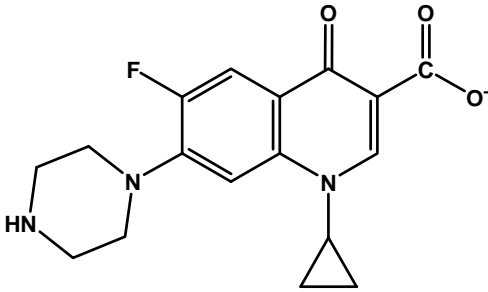
BUT ABC transporters also expel antibiotics!

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

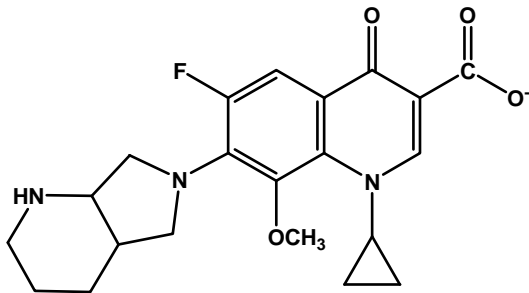
Two fluoroquinolones...

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

Ciprofloxacin (CIP)



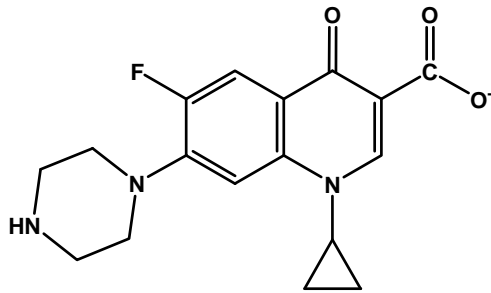
Moxifloxacin (MXF)



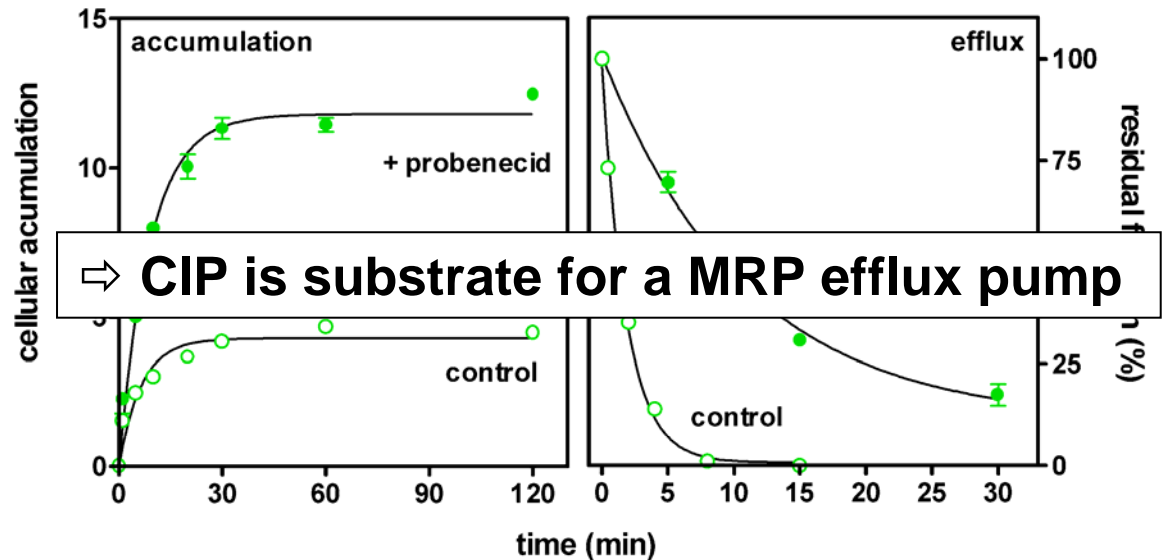
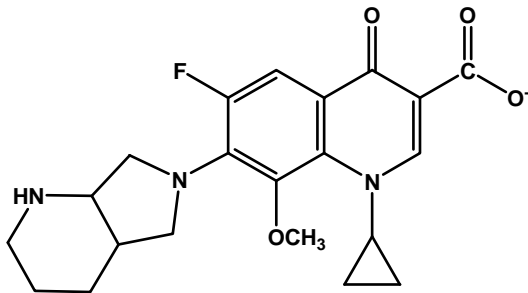
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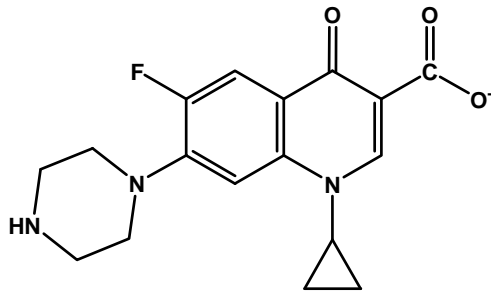
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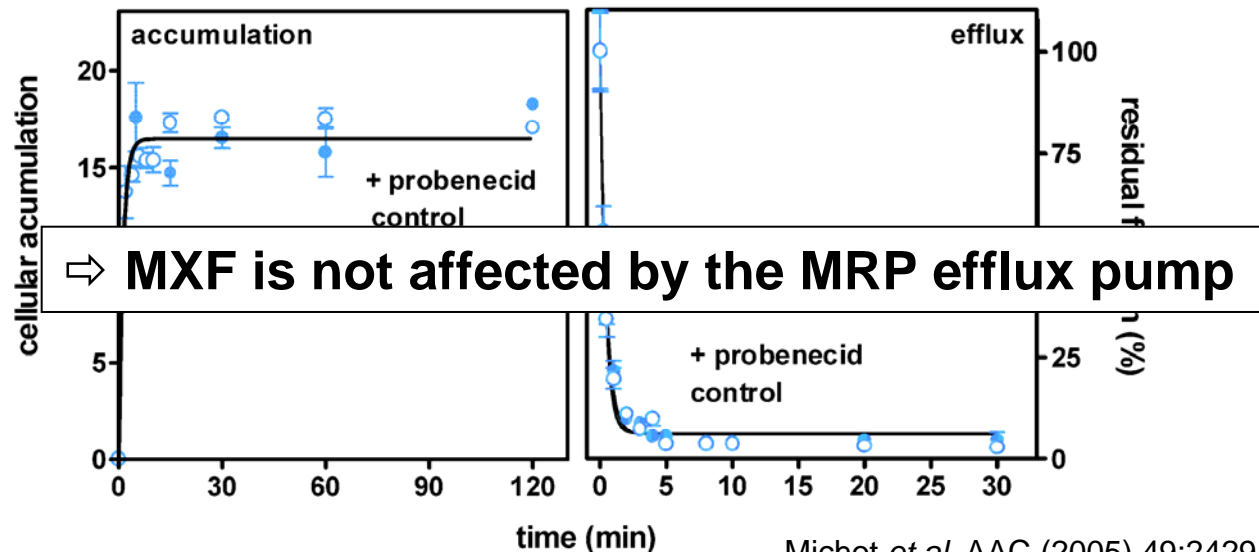
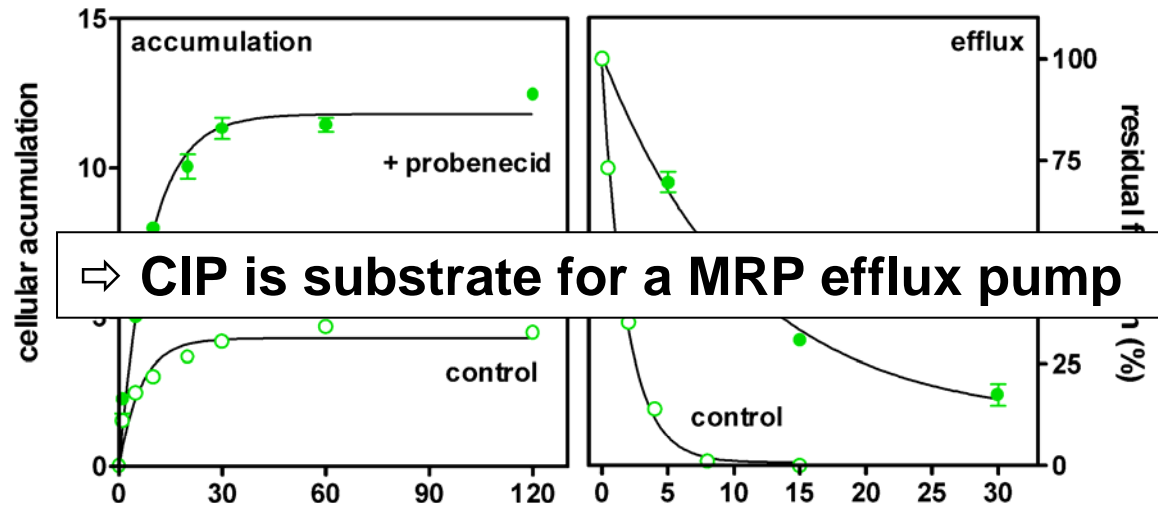
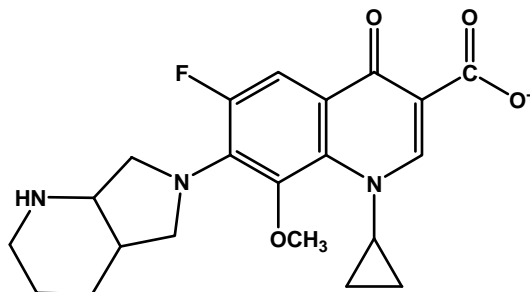
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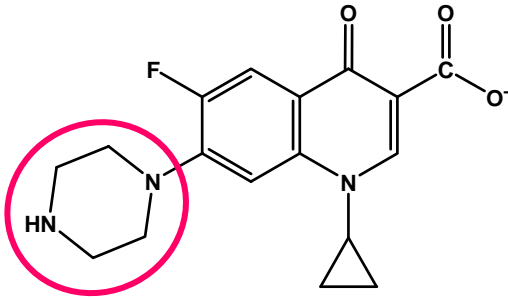
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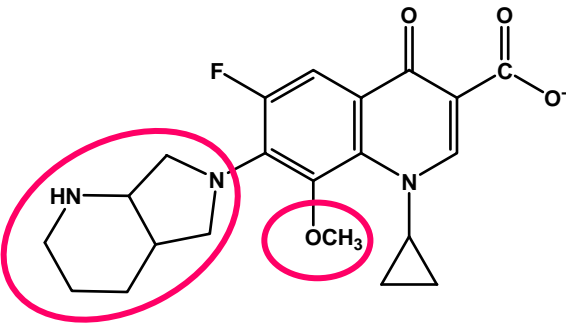
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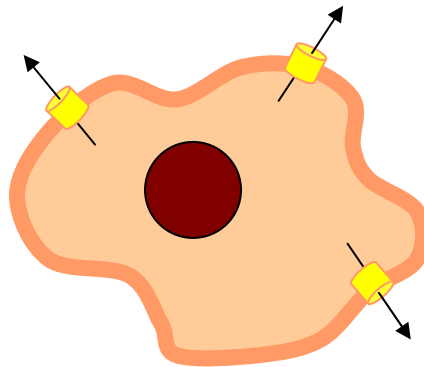
Moxifloxacin (MXF)



... 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

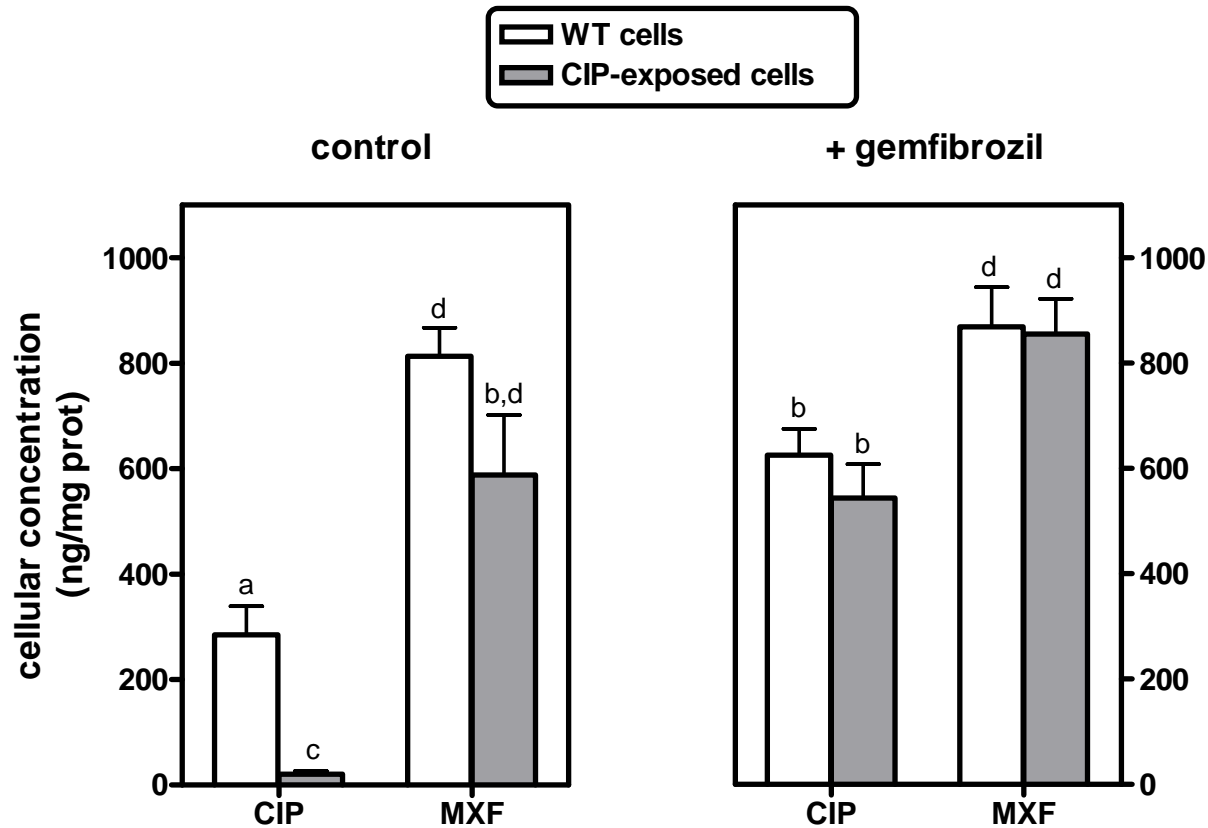
Chronic exposure to increasing drug concentrations of CIP or MXF

???

Selected macrophages: resistant to CIP or MXF?

CIP-exposed macrophages

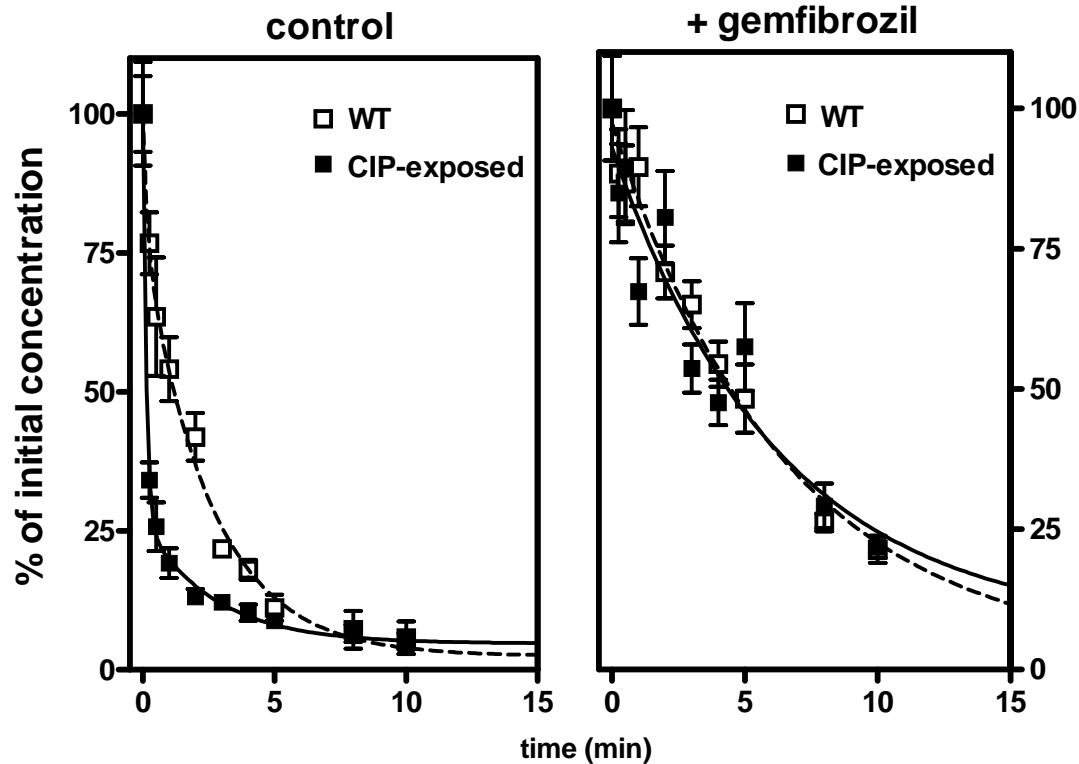
Fluoroquinolones accumulation



- **CIP** accumulation \searrow in **CIP-exposed** macrophages and \nearrow with gemfibrozil
 - **MXF** accumulation does not change significantly \pm gemfibrozil
- \Rightarrow Selection of **CIP-RESISTANT** macrophages

CIP-exposed macrophages

CIP efflux



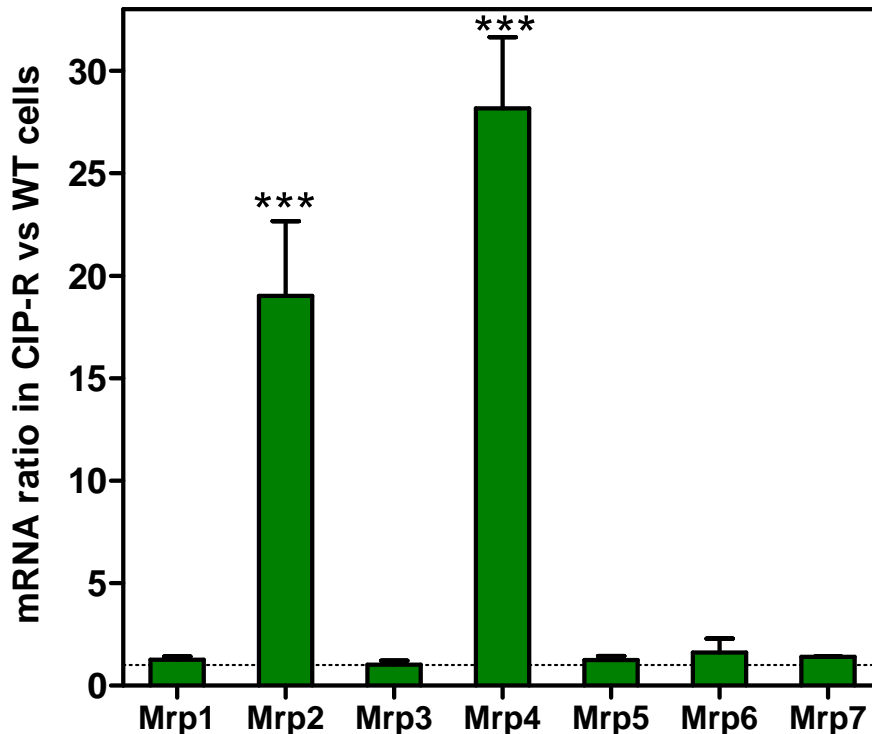
	$T_{1/2}$ CIP (min)	
	control	+ gemfibrozil
WT	1.75	4.49
CIP-exposed	0.09	4.35

☞ Which MRP efflux pump is involved?

CIP-exposed macrophages

Mrp expression

- ◆ Quantification of mRNA by real-time PCR

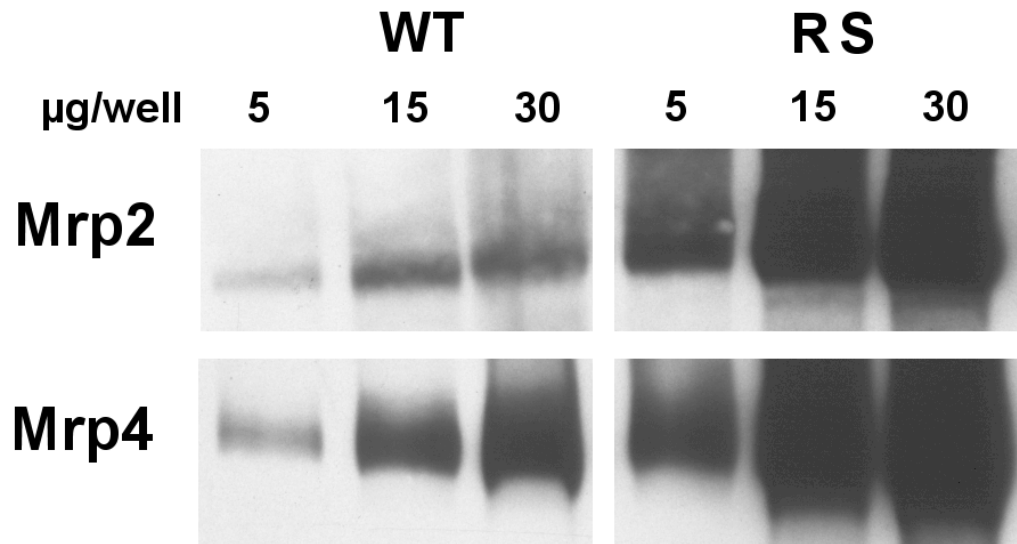


⇒ Overexpression of *Mrp2* and *Mrp4* in CIP-exposed macrophages

CIP-exposed macrophages

Mrp expression

- ◆ Confirmation by Western Blot in membrane samples

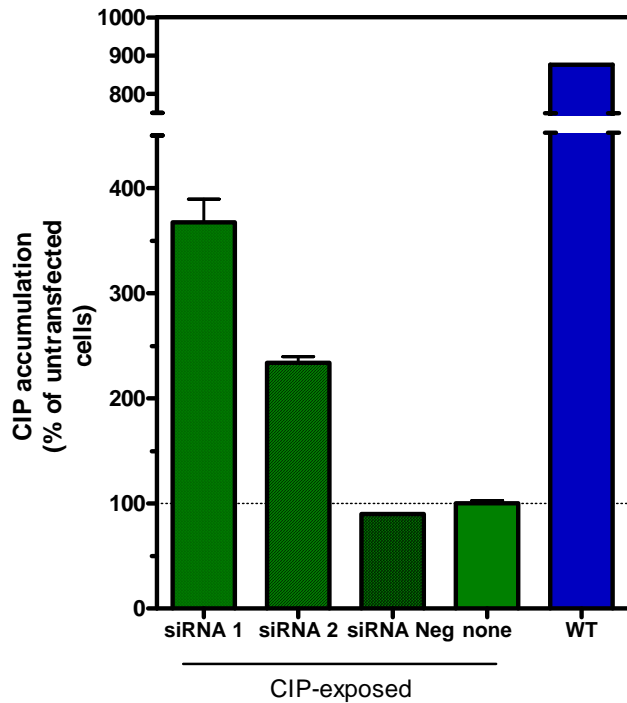
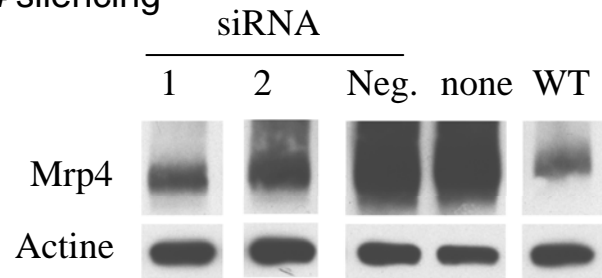


☞ Which one mediates **CIP** efflux?

CIP-exposed macrophages

Silencing of *Mrp4* and *Mrp2* by specific siRNA

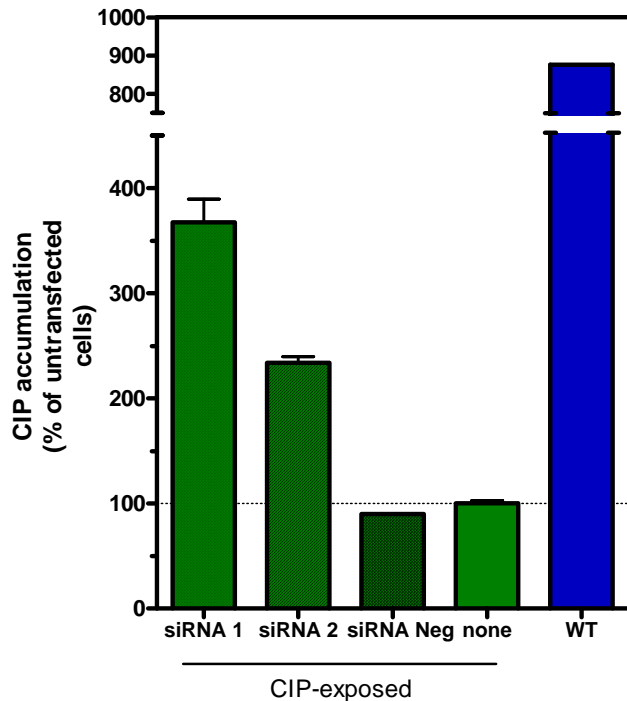
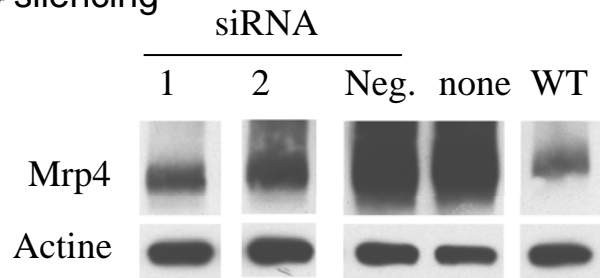
Mrp4 silencing



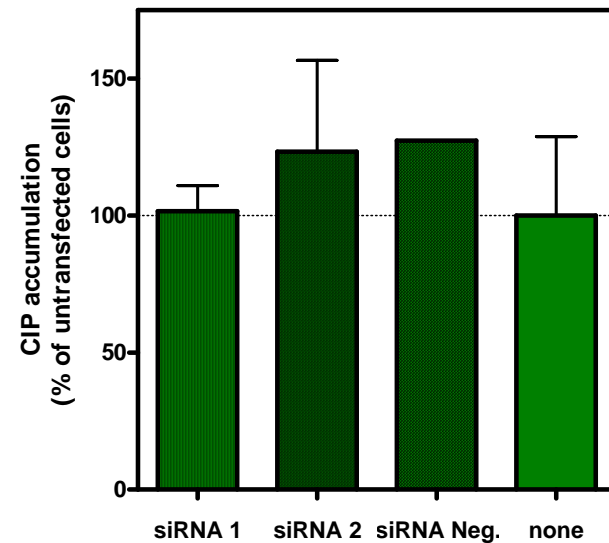
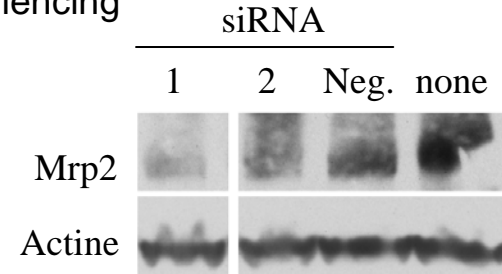
CIP-exposed macrophages

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Mrp4 silencing



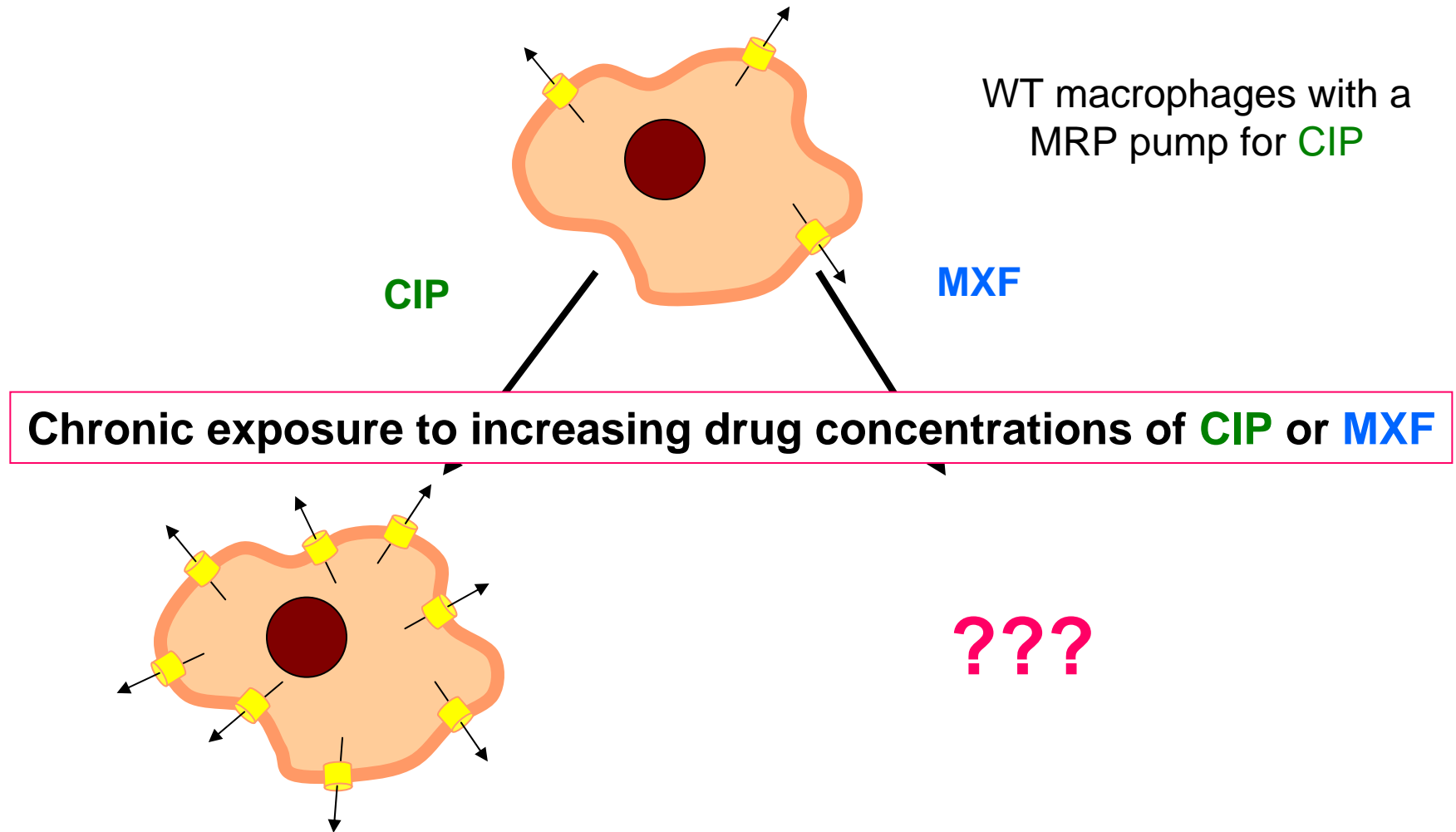
Mrp2 silencing



⇒ Only *Mrp4* is involved in CIP efflux in CIP-exposed macrophages

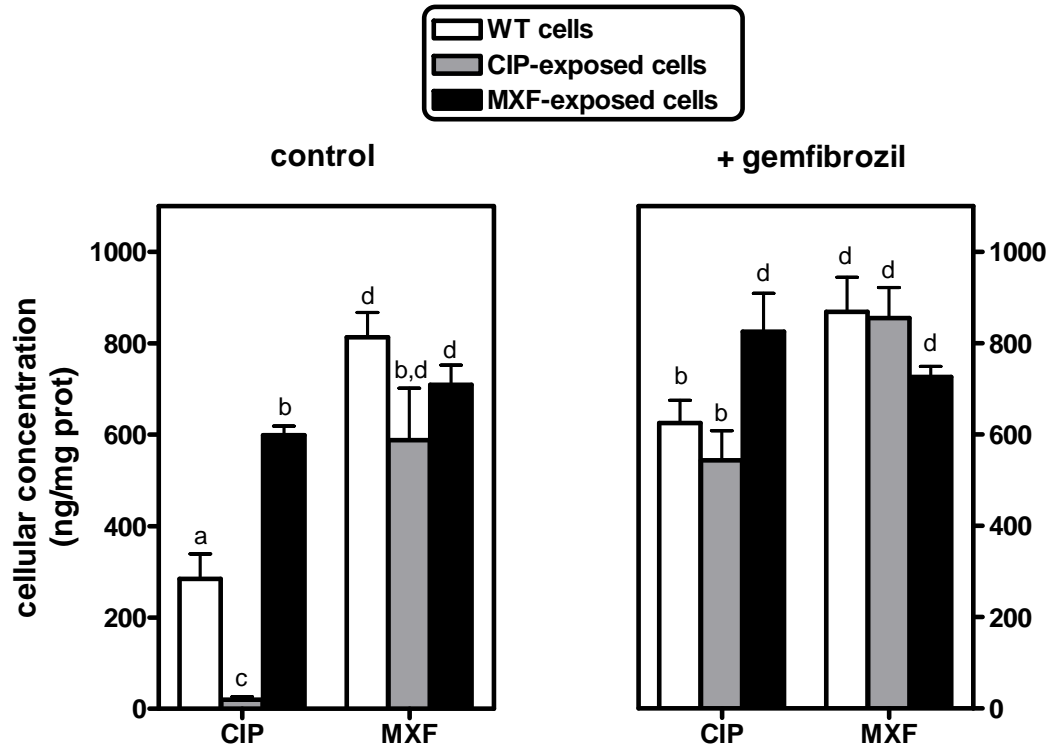
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MXF-exposed macrophages

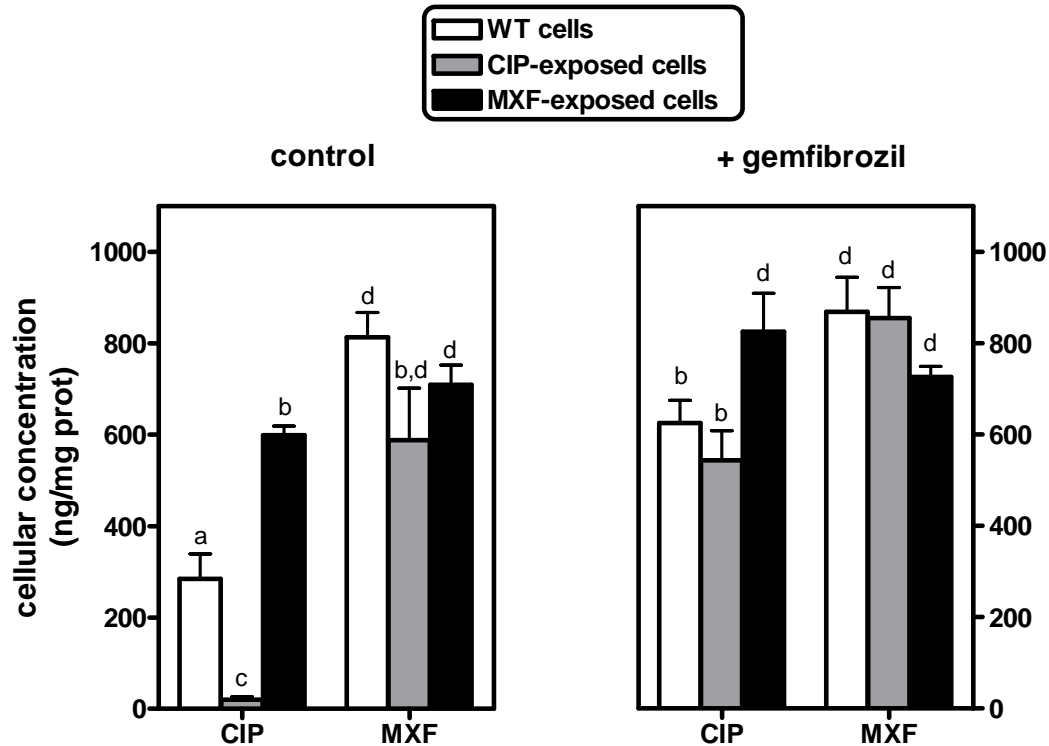
Fluoroquinolones accumulation



- **CIP** accumulation ↗ in **MXF-exposed** macrophages
- **MXF** accumulation does not change significantly

MXF-exposed macrophages

Fluoroquinolones accumulation



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

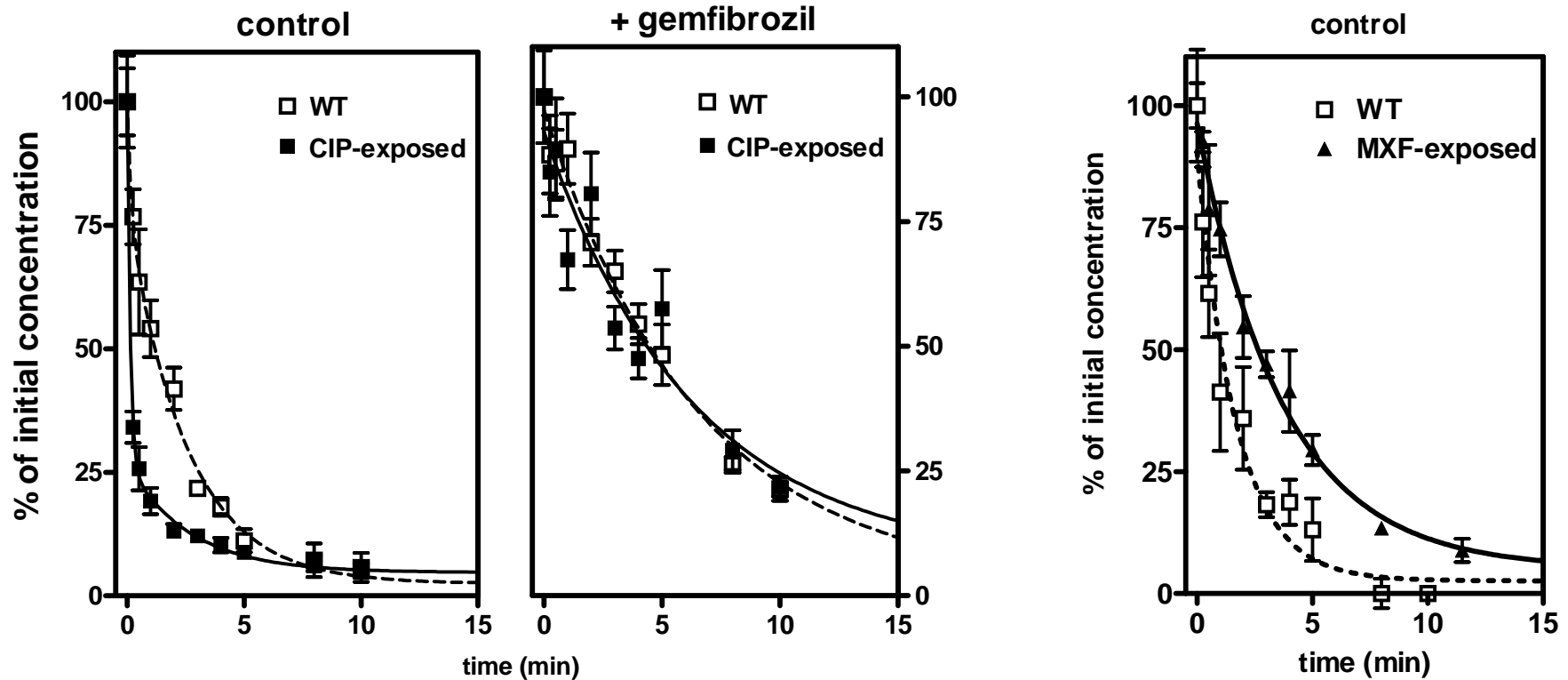
↪ Exposure to **MXF** induces an increase of **CIP** accumulation in macrophages

MXF-exposed macrophages

CIP efflux

In CIP-exposed macrophages

In MXF-exposed macrophages

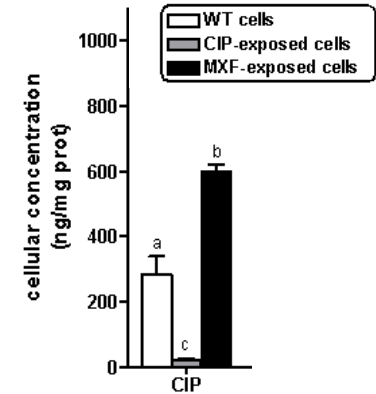


CIP efflux $T_{1/2}$: CIP-exposed < WT < MXF-exposed macrophages

CIP kinetics

$T_{1/2}$ for CIP

	Influx	Efflux
WT macrophages	3.6 min	1.2 min
CIP-exposed	nd	< 0.1 min
MXF-exposed	3.1 min	2.6 min

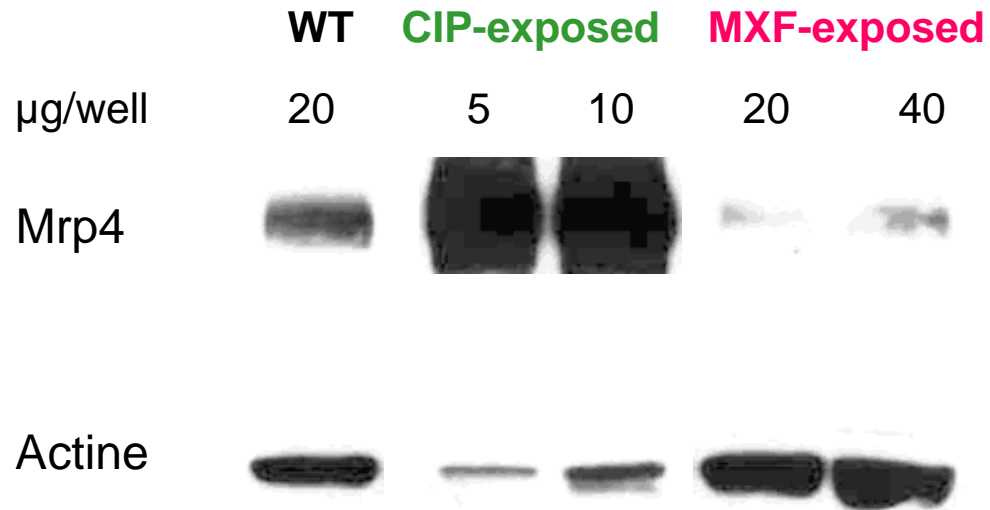


⇒ **Differences in CIP accumulation** are the consequences of a **faster (CIP-exposed macrophages)** or **slower (MXF-exposed macrophages) efflux** of the drug.

👉 Expression of **Mrp4** in **MXF-exposed** macrophages?

MRP content

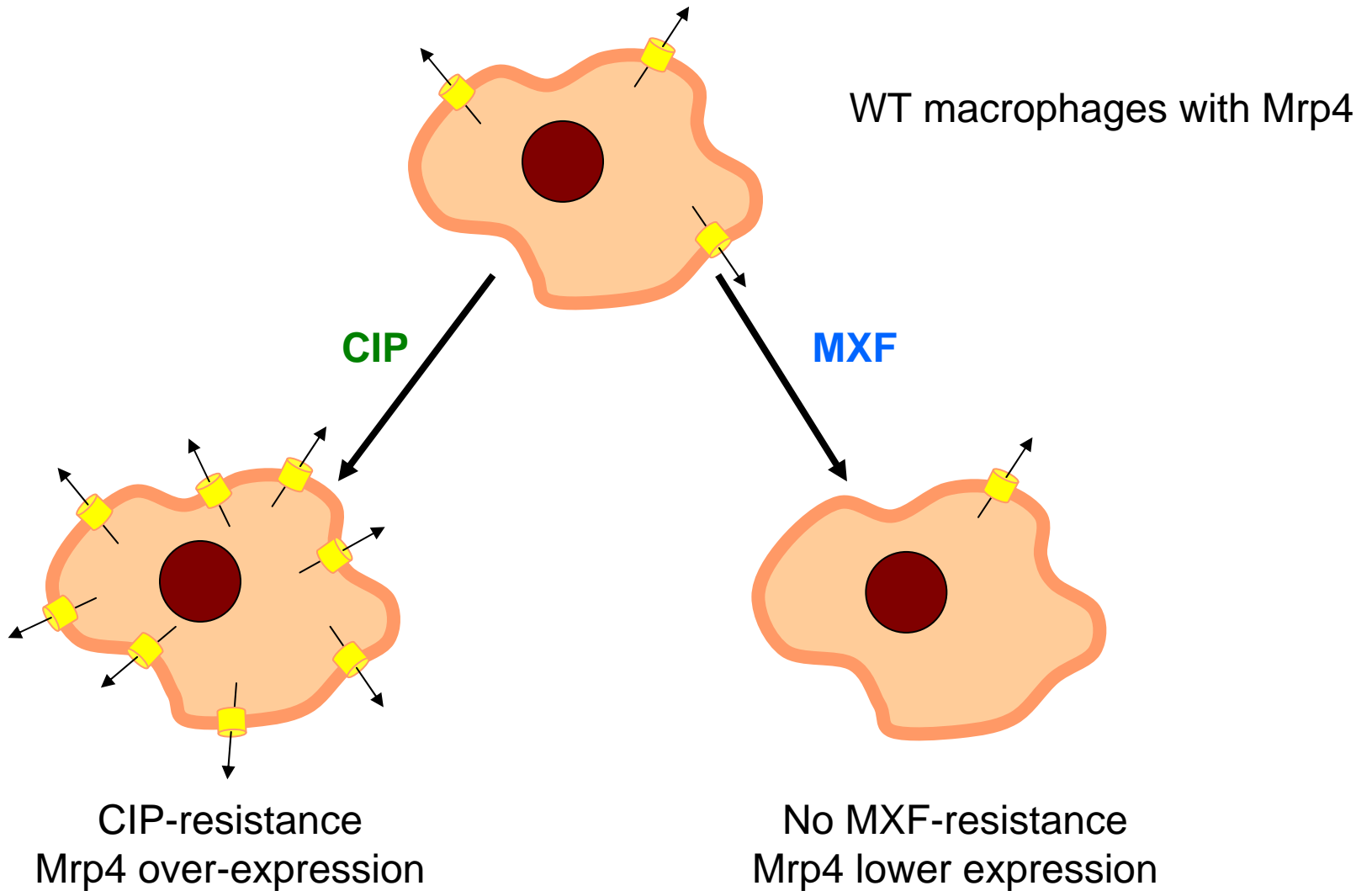
Western-Blot for Mrp4



⇒ **CIP-exposed** macrophages express more Mrp4

⇒ **MXF-exposed** macrophages express less Mrp4

Conclusions



Conclusions

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